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(54) Title: PLASMIN-RESISTANT STREPTOKINASE

(57) Abstract

The invention features modified streptokinase (SK) molecules which are resistant to plasmin cleavage including a recombinant fusion protein in which the amino terminus of SK was blocked with a peptide, a recombinant fusion protein in which an amino-terminal deleted SK was blocked with a peptide, and a mutated SK in which plasmin-cleavage sites were altered to render those sites resistant to enzymatic cleavage.

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PLASMIN-RESISTANT STREPTOKINASE

Background of the Invention

Streptokinase (SK), isolated from Group C streptococcus, is used as a plasminogen activator to accelerate the lysis of the coronary thrombi that cause heart attacks. However, SK is by itself inert and must combine with human plasminogen to form a catalytically-active SK-plasminogen activator complex (SK-PAC) which cleaves substrate plasminogen molecules. Studies of proteolytic fragments of SK and recombinant truncation mutants have defined regions of SK which are important for binding interactions with plasminogen in the construction of the activator complex. Through undefined molecular interactions, an active site appears in the plasminogen moiety of the SK-PAC (Buck et al., 1968, J. Biol. Chem. 246:209-246). The SK-PAC then generates the active enzyme plasmin by clipping substrate plasminogen molecules at the Arg560-Val bond (Robbins et al., 1987, In Colman et al., Hemostasis and thrombosis: basic principles and clinical practice, 2nd ed., Lippincott, Philadelphia, pp. 341-357).

Almost immediately after forming an active SK-PAC, the SK moiety is clipped to smaller molecular weight forms (Siefring and Castellino, 1976, J. Biol. Chem. 251:3913-3920; Markus et al., 1976, J. Biol. Chem. 251:6495-6504). Cleavage of SK markedly reduces the catalytic activity of the activator complex (Markus et al., 1976, *supra*). Enzymatic studies of SK fragments isolated after reacting with plasminogen at lower temperatures suggests that SK activity declines with progressive cleavage (Markus et al., 1976, *supra*).

Inactivation of SK in plasma as a result of plasmin cleavage reduces the therapeutic effectiveness of this plasminogen activation.

Summary of the Invention

The SK-derived compounds of the invention resist cleavage inactivation by plasmin, while retaining all or a substantial portion of the plasminogen-binding and catalytic activity of native SK. SK modified according to the invention is a more potent thrombolytic agent than native SK, and therefore, is a more useful therapeutic tool.

The invention features a compound containing (a) a plasminogen-binding fragment of SK and (b) a blocking group at the amino-terminus of the fragment. By the term "streptokinase" is meant an indirect plasminogen activator derived from streptococci. By the term "fragment" is meant a polypeptide containing less than or all of the native, full-length amino acid sequence of SK. SK may be recombinant or purified from streptococci, and the streptococci from which it is derived is preferably β-hemolytic. Alternatively, the streptokinase may be derived from an α-hemolytic streptococci. The streptococci from which SK is derived is preferably from Group C, e.g., *Streptococcus equisimilus*, however SK may also be derived from streptococci of Group A or Group G.

The compound is catalytically active and the rate of *in vitro* degradation in the presence of human plasminogen is at least two times slower than the rate of native, full-length mature SK protein derived from *Streptococcus equisimilus* (nSK), i.e., the time required from the addition of SK to plasminogen to the disappearance of the band on a Western blot corresponding to the uncleaved nSK. For example, the time required for the disappearance of uncleaved nSK is about 2 min., whereas the time for the disappearance of modified SK ranges from 7 min. to greater than 20 min. By the term "catalytically active" is meant it possesses the ability

of SK to interact with plasminogen to form a SK-PAC capable of activating plasminogen to plasmin. By the term "degradation" is meant the process by which SK is reduced by plasmin cleavage into lower molecular weight fragments. The rate of degradation is measured by the disappearance of a full-length recombinant SK as detected by immunoblotting using anti-SK antibodies.

The compound preferably contains the amino acid sequence of SEQ ID NO:4. The blocking group of the compound may be a peptide or a non-peptide blocking group which is located at the amino-terminus of the SK fragment. For example, a blocking group may be introduced by glycosylation or myristolization. Preferably, the blocking group is least one heterologous amino acid; more preferably, the blocking group is a heterologous peptide of two or more amino acids; and most preferably, the blocking group is a fragment of or all of maltose binding protein (MBP). By the term "heterologous" is meant an addition or substitution of one or more amino acids that is different from that found at the corresponding site in nSK.

The invention also includes a DNA, e.g., a DNA vector, containing a coding sequence which encodes the polypeptide portion of the compound of the invention, and a method of dissolving blood clots in a mammal by administering an effective amount of the compound. An effective amount of the compound is an amount which is effective in dissolving at least one blood clot in a patient.

The invention also features a plasminogen-binding fragment of SK which is catalytically active and the rate of *in vitro* degradation of which is at least two times slower than the rate of nSK in the presence of human plasminogen. The fragment preferably comprises at least 95% of the amino acid sequence of nSK; more preferably,

the fragment lacks one to five amino-terminal amino acids of nSK; more preferably, the fragment lacks one to ten amino-terminal amino acids; more preferably, the fragment lacks 1-24 amino acids. In a preferred embodiment, the fragment consists of amino acids 14-414 of nSK (SEQ ID NO:4). A fragment consisting of amino acids 14-414 of nSK (SEQ ID NO:4) may also contain at least one or more mutations selected from the group consisting of K36A, R45A, K51A, K59A, K61A, K147A, K333, R232A, K257A, K298A, K309A, R234A, R363A, K386A, K372A, R388A, R394A, and R401A.

The invention also includes an SK polypeptide which is catalytically active and the rate of *in vitro* degradation of which is at least two times slower compared to the rate of nSK. By "polypeptide" is meant a chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation). Preferably, the polypeptide consists of the amino acid sequence of nSK in which at least one potential plasmin cleavage site has been mutated to render it resistant to plasmin cleavage. More preferably, the polypeptide contains one or more mutations selected from the group consisting of R10A, K36A, R45A, K51A, K59A, K61A, K147A, K333, R232A, K257A, K298A, K309A, R234A, R363A, K386A, K372A, R388A, R394A, and R401A. Most preferably, the fragment is rSK5mut (SEQ ID NO:17), which contains the mutations, R10A, R36A, R45A, R51A, and R59A or rSK6mut, which contains the mutations R10A, R36A, R45A, R51A, R59A, and K386A (SEQ ID NO:18). The invention also includes a DNA containing a coding sequence encoding the SK polypeptide of the invention and a method of dissolving blood clots in a mammal by administering to the mammal an effective amount of the SK polypeptide of the invention.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings will first be described.

Drawings

Fig. 1 is a photograph of a Western blot showing purification of a fusion protein with maltose binding protein linked to the amino terminus nSK (rSK), a fusion protein with MBP linked to the amino terminus of nSK in which the amino terminal 13 amino acids of nSK were deleted (rSKΔ14), and rSK5mut.

Fig. 2 is a graph showing plasminogen activation by nSK, rSK, and rSKΔ14.

Fig. 3 is a photograph of a Western blot showing plasmin cleavage of nSK.

Fig. 4 is a photograph of a Western blot showing plasmin cleavage of rSK (0-20 min.).

Fig. 5 is a photograph of a Western blot showing plasmin cleavage of rSKΔ14.

Fig. 6 is a photograph of a Western blot showing plasmin cleavage of rSK5mut.

Fig. 7 is a photograph of a Western blot showing comparative plasmin cleavage of rSK, rSKΔ14, nSK, and rSK5mut.

Modification of SK to render it resistant to degradation by plasmin

Within seconds, binding of SK to plasminogen to form SK-PAC, nSK is rapidly degraded at its amino terminus by plasmin. Through the process of degradation, plasmin limits the thrombolytic efficacy of nSK. According to the invention, SK can be modified in three different ways to render it resistant to plasmin

cleavage: (1) by blocking the amino terminus of nSK, e.g., with a heterologous peptide; (2) by deleting one or more amino terminal amino acids from nSK; and (3) by altering plasmin cleavage sites throughout nSK to render them resistant to plasmin cleavage. In one example, a recombinant fusion protein was made in which the amino terminus of nSK was tethered in peptide linkage to MBP (rSK). In another example, a recombinant fusion protein was made in which the MBP was linked to the amino terminus of nSK, the first 13 amino acids of which were deleted. In the third example, the nSK amino acid sequence was mutated at plasmin-cleavage sites to render those sites resistant to enzymatic cleavage, e.g., in the mutant rSK5mut, the K or R residue in five potential plasmin cleavage sites were changed to A residues. In each case, plasmin cleavage yielded catalytically active plasmin cleavage products, but the rate of degradation was markedly reduced compared to that of nSK. In addition to affecting the rate of degradation, mutation of plasmin cleavage sites also significantly decreases the K_m of amidolytic activity, which leads to greater catalytic efficiency.

Therapeutic Applications

The compounds of the invention can be used to lyse blood clots in a mammal. The compounds can be administered by any standard route including intraperitoneally, intramuscularly, subcutaneously, or intravenously. It is expected that the preferred route of administration will be intravenous. The compounds can be administered systemically to the bloodstream as well as locally within the blood vessel at the site of clot formation. Since the compounds of the invention are timed-release, they can be administered in a single dose rather than by continuous infusion.

As is well known in the medical arts, dosages for any one patient depends on many factors, including the patients general health, sex, size, body surface area, age, as well as the particular compound to be administered, time and route of administration, and other drugs being administered concurrently. Dosages for the compounds of the invention will vary, but a preferred dosage for administration to human patients is approximately 20,000 units per kg of body weight (units of SK are defined in Bulletin. World. Health. Org., 1965, 33:235). Determination of correct dosage for a given application is well within the abilities of one of ordinary skill in the art of pharmacology. Optimal dosage may be adjusted according to the condition of the patient and response of the patient to therapy.

EXAMPLE 1: Modification of the amino terminus of streptokinase modulates the appearance of the active site in the SK-PAC

To examine the functional role of the amino terminus of SK in the SK-PAC, the amino terminus of SK was recombinantly modified by partial deletion of amino-terminal amino acids or by tethering of the amino terminus with a blocking group, e.g., a heterologous peptide. Functional activity of the modified SK was evaluated by measuring (1) the rate of plasminogen activation by SK-PAC, (2) the amidolytic activity of the SK-PAC, and (3) the plasmin-mediated degradation of SK in the SK-PAC.

Cloning, Expression and Purification of Streptokinase.

The SK gene (Malke et al., 1985, Gene 34:357-362) was cloned from *Streptococcus equisimilis* by the polymerase chain reaction (PCR), sequenced (U.S. Biochemicals, Cleveland, Ohio; Sanger et al., 1977, Proc.

Natl. Acad. Sci USA 74:5463) and subcloned into the pMAL vector for bacterial expression (New England Biolabs, Beverly, MA) using known methods, e.g., Reed et al., 1993, J. Immunol. 150:4407-4415; Reed et al., 1993, Circulation 88:Abstract I-615). The expressed SK gene formed a fusion protein with maltose binding protein at its amino terminus (rSK). Restriction digestion of the SK gene with *Hinc II* removed the nucleotides encoding the amino terminal 13 amino acids of SK to produce deletion mutant, rSK$_{\Delta}$14. These recombinant SK fusion proteins were purified by affinity chromatography on an amylose resin (New England Biolabs, Beverly, MA) as described by the supplier. The purity of the recombinant SK fusion proteins was assessed by SDS-PAGE (Laemmli, 1970, Nature 227:680-685). For some experiments, the SK fusion proteins were cut with factor Xa (Maina et al., 1988, Gene 74:365) and the MBP portion of the fusion protein removed by affinity chromatography on an amylose resin.

After purification, the relative concentrations of the recombinant SKs were determined by comparative radioimmunoassay (RIA) using anti-SK monoclonal antibodies. Wells of a microtiter plate were coated with various concentrations of nSK (0, 2.5, 5, 10, 20, and 40 μg/mL) or different dilutions of the recombinant SKs, rSK$_{\Delta}$14 and rSK5mut. After nonspecific binding sites had been blocked with 1% bovine serum albumin, anti-SK monoclonal antibodies were added to each well in duplicate. After a 1-h incubation, the wells were washed and probed with ^{125}I goat anti-mouse antibody (Cappel Organon Teknika, Durham, NC) for 1 h. After another wash, the amount of bound antibody was determined by gamma counting. A standard curve relating antibody binding (cpm) to nSK concentration was derived and the concentration of each recombinant SK was determined by reference to the standard curve.

Plasminogen Activation by recombinant SKs.

Studies of the time-related activity of different SKs were carried out by mixing Glu-plasminogen (333 nM; American Diagnostica, Greenwich, CT) in a quartz cuvette with S2251 (0.5 mM; H-D-valyl-L-leucyl-L-lysine-p-nitroanilide dihydrochloride, Chromogenix, Sweden) at 21°C or 37°C and then adding purified nSK, rSK, or rSKΔ14 (16.7 nM). Absorption at 405 nanometers was continuously monitored in a Hewlett-Packard diode array spectrophotometer.

Active Site Titration

The development of an active site in the SK-PAC was monitored using standard methods. Plasminogen (8.5 μg; Sigma, St. Louis, MO) was added to a quartz cuvette containing 2 ml of filtered buffer (50 mM, 100 mM NaCl, pH 7.4) and 1 mM of the fluorogenic substrate 4-methylumbelliferyl p-guanidinobenzoate (Sigma, St. Louis, MO) thermostatically maintained at 25°C. The emission at 445 nanometers (excitation at 365 nanometers) was continuously monitored in a Hitachi 2000 fluorescence spectrophotometer. After ~200 seconds of observation, rSK was added, and the reaction was recorded for a total of 2000 seconds.

Kinetic Assays of the SK-PAC

The amidase kinetic parameters of nSK, rSK and rSKΔ14 were studied using a paranitroanilide substrate (S2251, H-D-valyl-L-leucyl-L-lysine-p-nitroanilide dihydrochloride, Chromogenix, Sweden) using known methods, e.g., Wohl R. et al., 1980., Biochim. et Biophys. Acta 745:20-31). The recombinant SK proteins and Glu-plasminogen were mixed together and incubated for 5 min. (nSK and rSK) or 20 min (rSKΔ14) at 37°C. The mixture was then transferred to a quartz cuvette

containing assay buffer (50 mM Tris, 100 mM NaCl, pH 7.4) and various concentrations of S2251 (100-800 μM) added. The cuvette was thermostatically regulated at 37°C. The change in absorbance was monitored at 404 nM for 10 min. at 37°C, and the data were transformed to Linewaever-Burke plots to determine the K_m and V_{max}.

Studies of the degradation of SK by plasmin

The time-related proteolysis of nSK, rSK, rSKΔ14, and rSK5mut was studied by immunoblotting. nSK (1 μg) or recombinant SKs (2 μg) were mixed together with purified human Glu-plasminogen (40 μgs; American Diagnositica, 98% Glu-type plasminogen) for 0-20 min. The amount of human plasminogen present is typically in excess of the amount of SK. At various time points, an aliquot (5 μl) was removed and plunged into boiling water to stop the reaction. The samples were then electrophoresed on 10% SDS-polyacrylamide under reducing conditions and electrophoretically transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Nonspecific binding sites were blocked with 5% nonfat milk for 1 hr. The blots were incubated with pooled monoclonal antibodies specific for SK overnight at 4°C. The blots were washed and incubated for 1 hr. with ^{125}I-goat antimouse antibody (~1,000,000 cpm; Cappel Organon Teknika, Durham, NC) which had been labelled using the Iodogen labelling method known in the art. After washing, the blots were exposed to Kodak X-O-mat film (Rochester, NY) at -70°C.

Amino-terminal modification of SK

SK was produced as a fusion protein with MBP at its amino terminus (rSK), the amino acid sequence of which is shown in Table 1. A mutant lacking the first 13 amino acids of SK was also produced as a fusion protein (rSKΔ14), the amino acid sequence of which is shown in Table 2. The amino acid sequence of nSK is shown in Table 3, and the amino acid sequence of SKΔ14 is shown in Table 4. The sequence of both rSK and rSKΔ14 suggested that they could be cleaved at the fusion protein junction by factor Xa. The production of the rSK proteins in *E. coli* was induced by IPTG. Recombinant SK proteins were purified from bacterial lysates by affinity chromatography. As shown in Fig. 1, the proteins migrated at the predicted molecular size (rSK: 89 kDa, rSKΔ14: 87 kDa).

Table 1: rSK

MKTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDG
PDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEAL
SLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFK
YENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTIN
GPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYL
LTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWY
AVRTAVINAASGRQTVDEALKDAQTNSSSVPGRGSIEGRIAGPEWLLDRPSVNNSQL
VVSVAGTVEGTNQDISLKFFEIDLTSRPAHGGKTEQGLSPKSKPFATDSGAMSHKLE
KADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVTLPTQPV
QEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKLLKTLAI
GDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEFTYRVKN
REQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIKYVDVDT
NELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVEDNHDDT
NRIITVYMGKRPEGENASYHLAYDKDRYTEEEREVYSYLRYTGTPIPDNPNDKNNSQ
LVVSVAGTVEGTNQDISLKFFEIDLTSRPAHGGKTEQGLSPKSKPFATDSGAMSHKL
EKADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVTLPTQP
VQEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKLLKTLA

IGDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEFTYRVK
NREQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIKYVDVD
TNELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVEDNHDD
TNRIITVYMGKRPEGENASYHLAYDKDRYTEEEREVYSYLRYTGTPIPDNPNDK
(SEQ ID NO:1)

Table 2: rSKΔ14

MKTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDG
PDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEAL
SLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFK
YENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTIN
GPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYL
LTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWY
AVRTAVINAASGRQTVDEALKDAQTNSSSVPGRGSIEGRNNSQLVVSVAGTVEGTNQ
DISLKFFEIDLTSRPAHGGKTEQGLSPKSKPFATDSGAMSHKLEKADLLKAIQEQLI
ANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVTLPTQPVQEFLLSGHVRVRY
KEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKLLKTLAIGDTITSQELLAQA
QSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEFTYRVKNREQAYRINKKSGL
NEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIKYVDVDTNELLKSEQLLTAS
ERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVEDNHDDTNRIITVYMGKRPE
GENASYHLAYDKDRYTEEEREVYSYLRYTGTPIPDNPNDKNNSQLVVSVAGTVEGTN
QDISLKFFEIDLTSRPAHGGKTEQGLSPKSKPFATDSGAMSHKLEKADLLKAIQEQL
IANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVTLPTQPVQEFLLSGHVRVR
YKEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKLLKTLAIGDTITSQELLAQ
AQSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEFTYRVKNREQAYRINKKSG
LNEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIKYVDVDTNELLKSEQLLTA
SERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVEDNHDDTNRIITVYMGKRP
EGENASYHLAYDKDRYTEEEREVYSYLRYTGTPIPDNPNDK (SEQ ID NO:2)

Table 3: nSK

IAGPEWLLDRPSVNNSQLVVSVAGTVEGTNQDISLKFFEIDLTSRPAHGGKTEQGLS
PKSKPFATDSGAMSHKLEKADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRNG
KVYFADKDGSVTLPTQPVQEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNPD
DDFRPGLKDTKLLKTLAIGDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDND
IFRTILPMDQEFTYRVKNREQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYDP

FDRSHLKLFTIKYVDVDTNELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDAF
GIMDYTLTGKVEDNHDDTNRIITVYMGKRPEGENASYHLAYDKDRYTEEEREVYSYL
RYTGTPIPDNPNDKNNSQLVVSVAGTVEGTNQDISLKFFEIDLTSRPAHGGKTEQGL
SPKSKPFATDSGAMSHKLEKADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRN
GKVYFADKDGSVTLPTQPVQEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNP
DDDFRPGLKDTKLLKTLAIGDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDN
DIFRTILPMDQEFTYRVKNREQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYD
PFDRSHLKLFTIKYVDVDTNELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDA
FGIMDYTLTGKVEDNHDDTNRIITVYMGKRPEGENASYHLAYDKDRYTEEEREVYSY
LRYTGTPIPDNPNDK (SEQ ID NO:3)

Table 4: SKΔ14

NNSQLVVSVAGTVEGTNQDISLKFFEIDLTSRPAHGGKTEQGLSPKSKPFATDSGAM
SHKLEKADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVTL
PTQPVQEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKLL
KTLAIGDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEFT
YRVKNREQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIKY
VDVDTNELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVED
NHDDTNRIITVYMGKRPEGENASYHLAYDKDRYTEEEREVYSYLRYTGTPIPDNPND
KNNSQLVVSVAGTVEGTNQDISLKFFEIDLTSRPAHGGKTEQGLSPKSKPFATDSGA
MSHKLEKADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVT
LPTQPVQEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKL
LKTLAIGDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEF
TYRVKNREQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIK
YVDVDTNELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVE
DNHDDTNRIITVYMGKRPEGENASYHLAYDKDRYTEEEREVYSYLRYTGTPIPDNPN
DK (SEQ ID NO:4)

Functional activity of recombinant SKs

To compare the function of SK, rSK and rSKΔ14, the rate of plasminogen activation by these proteins was examined at 21°C. nSK rapidly activated plasminogen with a minimal lag phase, i.e., less than 50 sec. (see Fig. 2). However, when expressed as a fusion protein, rSK showed a lag phase in plasminogen activation of

approximately 150 sec. (see Fig. 2). When expressed as a fusion protein lacking the amino terminal 13 amino acids, rSKΔ14 also showed a marked delay in time to activation os approximately 250 sec. (see Fig. 2). The lag phase refers to the time required for the reaction to the exponential phase of activity, e.g, full catalytic activity.

Plasmin cleavage products

Since nSK is known to be cleaved by plasmin after formation of the SK-PAC, the rate of cleavage of rSK and rSKΔ14 was examined after various times of incubation with Glu-plasminogen. In these experiments, SK was mixed with an excess of plasminogen for various amounts of time and the resulting cleavage of SK was determined by immunoblotting with monoclonal anti-SK antibodies. nSK was found to be rapidly degraded by plasmin within 30 secs to four lower molecular weight species, predominantly a ~36 kDa fragment (see Figs. 3 and 7). In contrast, the degradation of rSK was slower, yielding a fragment of 47 kDa (identical in size to nSK), first appearing at 1 min. A pattern of smaller SK fragments similar to that observed with nSK developed thereafter. After 5 min., a ~36 kDa SK fragment similar to that seen after nSK cleavage was found to be the major remnant from rSK (see Figs. 4 and 7). Other lower molecular weight SK fragments, e.g., ~28 kDa, were also evident as cleavage products of nSK, and at later time points, of rSK. Plasmin cleavage products of rSKΔ14 are shown in Fig. 5.

Amino-terminal deletion of SK

The amino terminal 13 residues of SK are highly conserved among the SKs produced by different groups of streptococci. In addition, this region constitutes a major epitope for both murine and human antibodies

against SK. Removal of the amino-terminal 13 amino acids from nSK resulted in a further increase in the lag phase of plasminogen activation by rSKΔ14, as compared to rSK. This lag phase was marked at 21°C, but shortened significantly when the temperature was raised to 37°C. Active site titration experiments indicate that removal of the amino terminus further delays the generation of the active site in the rSKΔ14.

Advantages of amino-terminally modified SK

At 37°C, and *in vivo*, nSK rapidly forms an active site with plasminogen. The kinetics of this activation has been regarded as suboptimal for therapy because plasmin is rapidly activated in one large burst *in vivo*. To overcome the explosive activation of plasminogen, an acylated SK-PAC (APSAC) made from SK and purified human plasminogen has been created *in vitro* (Ferres, 1987, Drugs 33 (Suppl. 3) 33). This approach permits APSAC to be given as a single bolus *in vivo* because continuous deacylation of the active site proceeds with a half-life of 40 mins (Staniforth et al., 1983, Eur. J. Clin. Pharmacol. 24:751). A limitation of this approach is that the rate of appearance of the active SK-PAC is determined by the rate of deacylation and can not be otherwise modulated.

In contrast, recombinant modification of the amino terminus of SK, either by expression as a fusion protein, or by deletion of the amino terminus, can predictably alter the rate of active site generation. For example, the extent to which the rate of degradation is reduced compared to nSK is directly proportional to the number of deleted amino-terminal amino acids (up to 13 amino acids). Other advantages of the SK-derived compounds of the invention include a short half-life: 2-4 min.; safety: the compounds of the invention are not made from

human blood products; and cost-effectiveness: the compounds of the invention are recombinantly produced. The activity of the compounds is timed-released, therefore they can be administered in a single dose. The time required to achieve SK activity may also be modified depending on the number of amino-terminal amino acids removed from the nSK, i.e., length of time required is directly proportional to the number of amino acids deleted. In this manner, the timed-release activity of SK can be customized to suit the specific clinical application or patient to be treated. Thus, the compounds of the invention are improved clinical reagents because, using modified rSKs, an active SK-PAC can be generated at a rate consistent with best thrombolytic results.

EXAMPLE 2: Site-directed streptokinase mutants resist cleavage and degradation by plasmin

To examine the effects of cleavage on the activity of SK, site-directed mutations of R or L to A at putative plasmin or trypsin cleavage sites in the amino and carboxy terminus of SK were generated. The cleavage rate of these recombinant SKs were then examined. The catalytic function of rSKs with these specific mutations was also evaluated.

SK cloning and mutation by overlap extension

The SK gene was cloned from Group C *Streptococcus equisimilis* as described above. A series of mutations was performed in the amino terminus of SK to replace R or K residues with an A residue at putative plasmin cleavage sites. In addition, a single K to A mutation was constructed for K386 in the carboxy terminus of SK. PCR primers were used to produce site-directed mutations by the overlap extension method. For example, using nSK in

the pMAL vector as a template, PCR was performed using a primer corresponding to the mal E sequence of the pMALc vector and the SK 10 AS primer. At the same time the SK 10 S primer was used in a PCR reaction with a SK 36 AS primer. The PCR products were purified on a low-melt agarose gel and used in an overlap PCR reaction. The overlapped product was then further amplified using the mal E primer and the SK 36 AS primer. In a similar fashion, the primers were used to construct mutations at the 45 and 51 position. The final overlap construct was between the 5′ overlapped mutated SK segment containing the mutations at SK 10, 36, 45, and 51 and the segment from 51 to 127. This overlapped fragment was then ligated into the pMALc nSK, replacing the wild type sequence, between restriction sites for *Kpn*I and *Afl*II. The SK 59 mutation was separately constructed and used to replace the wild type sequence between *Afl*II and *Mun*I. The mutation at residue 386 was similarly constructed and ligated into SK using a *Hind*III site. The mutated pMALcSKs were sequenced to verify the desired mutations.

Table 5. Primers for Mutation by Overlap Extension

Primer	Mutation	Primer Sequence	Restriction Site
SK 10 S	R->A	5'-GCTGCTAGACGCGCCATCTGTCAAC (SEQ ID NO:5)	HhaI
SK 10 AS		5'-TGGCGCGTCTAGCAGCCACTCAG (SEQ ID NO:6)	
SK 36 S	K->A	5'-CAAGACATTAGTCTGGCCTTTTTTGAAATCG (SEQ ID NO:7)	HaeIII
SK 36 AS		5'-GGCCAGACTAATGTCTTGATTCG (SEQ ID NO:8)	
SK 45 S	R->A	5'-CGATCTAACATCGGCGCCTGCTCATGG (SEQ ID NO:9)	NarI
SK 45 AS		5'-CGCCGATGTTAGATCGATTTC (SEQ ID NO:10)	
SK 51 S	K->A	5'-GCTCATGGAGGCGCCACAGAGGGC (SEQ ID NO:11)	NarI
SK 51 AS		5'-GGCGCCTCCATGAGCAGGTC (SEQ ID NO:12)	
SK 59 S	K->A	5'-GCTTAAGTCCGGCCTCAAAACCATTTGC (SEQ ID NO:13)	HaeIII
SK 59 AS		5'-TGAGGCCGGACTTAAGCCTTGCTC (SEQ ID NO:14)	
SK 386S	K->A	5'-GCCGATCGATATACCGAAGAAGAACGAG (SEQ ID NO:15)	ClaI
		5'-TATCGATCGGCATCATAGGCTAAATGATAGC (SEQ ID NO:16)	

Plasmin-resistant SK site mutants

The following plasmin cleavage sites can be mutated: R10A, K36A, R45A, K51A, K59A, K61A, K147A, K333, R232A, K257A, K298A, K309A, R234A, R363A, K386A, K372A, R388A, R394A, and R401A. Single mutants K59A, K386A, were made, and the multiple mutant containing R10A, K36A, R45A, K51A, and K59A (rSKmut5) was studied further. Purification of rSK5mut is shown in Fig. 1. Multiple mutant rSK6mut is identical to rSK5mut with the addition of another mutation at a carboxy-terminal potential plasmin cleavage site. This mutant contains the

following mutations: R10A, K36A, R45A, K51A, K59A and k386A.

The plasmin-resistant SK site mutants produce catalytically-active plasmin cleavage products which are larger than those generated from nSK (see Figs. 6 and 7). The rate of degradation of rSK5mut is also slower than that of nSK (see Figs. 6 and 7).

Kinetic studies were performed to examine the catalytic activity of the site mutants. Table 6 shows the results from kinetic studies for rSK5mut and Glu-plasminogen. These data show that mutation of plasmin cleavage sites significantly decreases the K_m of SK amidolytic activity leading to greater catalytic efficiency, and thus, greater therapeutic efficacy.

Table 6: Kinetic Parameters for recombinant SKs and Glu-Plasminogen

	K_m (μM)	K_{cat} (S^{-1})	k_{cat}/K_m ($\mu M^{-1}S^{-1}$)
nSK	248	56	0.226
rSK	152	42	0.276
rSKΔ14	533	51	0.096
rSK5mut	77	52	0.675

Table 7: rSK5mut

MKTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDG
PDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEAL
SLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFK
YENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTIN
GPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYL
LTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWY
AVRTAVINAASGRQTVDEALKDAQTNSSSVPGRGSIEGRIAGPEWLLDAPSVNNSQL
VVSVAGTVEGTNQDISLAFFEIDLTSAPAHGGATEQGLSPASKPFATDSGAMSHKLE
KADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVTLPTQPV
QEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKLLKTLAI
GDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEFTYRVKN
REQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIKYVDVDT
NELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVEDNHDDT
NRIITVYMGKRPEGENASYHLAYDKDRYTEEEREVYSYLRYTGTPIPDNPNDKNNSQ
LVVSVAGTVEGTNQDISLKFFEIDLTSRPAHGGKTEQGLSPKSKPFATDSGAMSHKL
EKADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVTLPTQP
VQEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKLLKTLA
IGDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEFTYRVK
NREQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIKYVDVD
TNELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVEDNHDD
TNRIITVYMGKRPEGENASYHLAYDKDRYTEEEREVYSYLRYTGTPIPDNPNDK
(SEQ ID NO:17)

Table 8: rSK6mut

MKTEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDG
PDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEAL
SLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFK
YENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTIN
GPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYL
LTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWY
AVRTAVINAASGRQTVDEALKDAQTNSSSVPGRGSIEGRIAGPEWLLDAPSVNNSQL
VVSVAGTVEGTNQDISLAFFEIDLTSAPAHGGATEQGLSPASKPFATDSGAMSHKLE
KADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVTLPTQPV
QEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKLLKTLAI
GDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEFTYRVKN
REQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIKYVDVDT
NELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVEDNHDDT
NRIITVYMGKRPEGENASYHLAYDADRYTEEEREVYSYLRYTGTPIPDNPNDKNNSQ
LVVSVAGTVEGTNQDISLKFFEIDLTSRPAHGGKTEQGLSPKSKPFATDSGAMSHKL
EKADLLKAIQEQLIANVHSNDDYFEVIDFASDATITDRNGKVYFADKDGSVTLPTQP
VQEFLLSGHVRVRYKEKPIQNQAKSVDVEYTVQFTPLNPDDDFRPGLKDTKLLKTLA
IGDTITSQELLAQAQSILNKNHPGYTIYERDSSIVTHDNDIFRTILPMDQEFTYRVK
NREQAYRINKKSGLNEEINNTDLISEKYYVLKKGEKPYDPFDRSHLKLFTIKYVDVD
TNELLKSEQLLTASERNLDFRDLYDPRDKAKLLYNNLDAFGIMDYTLTGKVEDNHDD
TNRIITVYMGKRPEGENASYHLAYDKDRYTEEEREVYSYLRYTGTPIPDNPNDK
(SEQ ID NO:18)

Table 9: DNA sequence of SK from S. equisimilus H46A

```
1     ctgcagctac ctgataccag gcatttccaa caaacatggt taaggccaaa
51    ccaaaatcac tttctagcgt tggcaagaga ccttcaagcg agcgcaagac
101   ctttattgaa gttgcttgtc gacataaaaa tgctgtttgg gttgtgctga
151   taggcaaaat gacctcaagc cctgcaatca tctgctggag caactcaact
201   aagtcagctg gtaaaacctg ctgatgattg aggtaaataa actgagaagt
251   ctcaaacagc tgaggggggat tgccctgatg atcaagcaaa taccgctgcc
301   aaggtgaccc tagcggctgc aagacctcat attgacccaa ccccacctca
351   agtaataagc gctctttttc ggataaacat gatttgggaa aatgcacata
401   ttggtcccct tctttgacac tcacccactc tttatctcct aacggatgag
451   ggcctacttg catctctgga aaatagtctt ttagctccat agccattcct
501   ttcatgacgg tctttaaacc attataacac atgactcttt atcacacagt
551   tcagtttgtt gtcagcacga ttttgtattt tctgcctttt taatcattaa
601   aactaaataa gggttattca tttttagcaa gaacattcaa ttaaatagct
651   atttatcgga atattaattt atgtttatgc taaaaaaggt attatttacc
701   ttttttcatt gtcattaaaa tatcatttta aaaaaatcaa taggttttta
751   tttgtgtctt taaaaccatt atgttattct aataatgggg attgaaactt
801   aacttttagg aggtttctat gaaaaattac ttatcttttg ggatgtttgc
851   actgctgttt gcactaacat ttggaacagt caattctgtc caagctattg
901   ctggacctga gtggctgcta gaccgtccat ctgtcaacaa cagccaatta
951   gttgttagcg ttgctggtac tgttgagggg acgaatcaag acattagtct
1001  taaatttttt gaaatcgatc taacatcacg acctgctcat aggaaaga
1051  cagagcaagg cttaagtcca aaatcaaaac catttgctac tgatagtggc
1101  gcgatgtcac ataaacttga gaaagctgac ttactaaagg ctattcaaga
1151  acaattgatc gctaacgtcc acagtaacga cgactacttt gaggtcattg
1201  attttgcaag cgatgcaacc attactgatc gaaacggcaa ggtctacttt
1251  gctgacaaag atggttcggt aaccttgccg acccaacctg tccaagaatt
1301  tttgctaagc ggacatgtgc gcgttagacc atataaagaa aaaccaatac
1351  aaaaccaagc gaaatctgtt gatgtggaat atactgtaca gtttactccc
1401  ttaaaccctg atgacgattt cagaccaggt ctcaaagata ctaagctatt
1451  gaaaaacacta gctatcggtg acaccatcac atctcaagaa ttactagctc
1501  aagcacaaag cattttaaac aaaaaccacc caggctatac gatttatgaa
1551  cgtgactcct caatcgtcac tcatgacaat gacattttcc gtacgatttt
1601  accaatggat caagagttta cttaccgtgt taaaaatcgg gaacaagctt
1651  ataggatcaa taaaaaatct ggtctgaatg aagaaataaa caacactgac
1701  ctgatctctg agaaatatta cgtccttaaa aaaggggaaa agccgtatga
1751  tccctttgat cgcagtcact tgaaactgtt caccatcaaa tacgttgatg
1801  tcgataccaa cgaattgcta aaaagtgagc agctcttaac agctagcgaa
1851  cgtaacttag acttcagaga tttatacgat cctcgtgata aggctaaact
1901  actctacaac aatctcgatg cttttggtat tatggactat accttaactg
1951  gaaaagtaga ggataatcac gatgacacca accgtatcat aaccgtttat
2001  atgggcaagc gacccgaagg agagaatgct agctatcatt tagcctatga
2051  taaagatcgt tataccgaag aagaacgaga agtttacagc tacctgcgtt
2101  atacagggac acctatacct gataacccta acgacaaata accacggtct
2151  tctaaaacga tgagattaac tgacaaaaaa agcaagcaac atgctatcaa
2201  cagttgcttg ctttttttcta acctcttagt tgtagagact agtgacattt
2251  cgtgtctaaa ataatcgtaa ctggtccatc attgatgaga ctaacctgca
2301  tatctgcccc aaaaacgcca cgctcaactg gcacaaaatc tgccaattgt
2351  tcattaaagc gatcataaaa ctggctagcc atatcagctt tgcagctcct
2401  gtaaaggctg ggcgatttcc cttttggtg tcagcataaa gggtaaattg
2451  cgacacagat aagatactac ccttgatgtc ttggatagac tgattcatct
```

2501 tgccatcagc atctgaaaaa atgcgcatgt tgactatttt tgcacagcgt
2551 aagccaaatc ttctgcag
(SEQ ID NO:19)

SK coding sequence spans nucleotides 819-2138; coding sequence of mature peptide spans nucleotides 897-2138.

Table 10: DNA sequence of MBP*

atgaaaactg aagaaggtaa actggtaatc tggattaacg gcgataaagg
ctataacggt ctcgctgaag tcggtaagaa attcgagaaa gataccggaa
ttaaagtcac cgttgagcat ccggataaac tggaagagaa attcccacag
gttgcggcaa ctggcgatgg ccctgacatt atcttctggg cacacgaccg
ctttggtggc tacgctcaat ctggcctgtt ggctgaaatc accccggaca
aagcgttcca ggacaagctg tatccgttta cctgggatgc cgtacgttac
aacggcaagc tgattgctta cccgatcgct gttgaagcgt tatcgctgat
ttataacaaa gatctgctgc cgaacccgcc aaaaacctgg gaagagatcc
cggcgctgga taaagaactg aaagcgaaag gtaagagcgc gctgatgttc
aacctgcaag aaccgtactt cacctggccg ctgattgctg ctgacggggg
ttatgcgttc aagtatgaaa acggcaagta cgacattaaa gacgtgggcg
tggataacgc tggcgcgaaa gcgggtctga ccttcctggt tgacctgatt
aaaaacaaac acatgaatgc agacaccgat tactccatcg cagaagctgc
ctttaataaa ggcgaaacag cgatgaccat caacggcccg tgggcatggt
ccaacatcga caccagcaaa gtgaattatg gtgtaacggt actgccgacc
ttcaagggtc aaccatccaa accgttcgtt ggcgtgctga gcgcaggtat
taacgccgcc agtccgaaca aagagctggc gaaagagttc ctcgaaaact
atctgctgac tgatgaaggt ctggaagcgg ttaataaaga caaaccgctg
ggtgccgtag cgctgaagtc ttacgaggaa gagttggcga aagatccacg
tattgccgcc accatggaaa acgcccagaa aggtgaaatc atgccgaaca
tcccgcagat gtccgctttc tggtatgccg tgcgtactgc ggtgatcaac
gccgccagcg gtcgtcagac tgtcgatgaa gccctgaaag acgcgcagac
taattcgagc tcggtacccg gccggggatc catcgagggt agg
(SEQ ID NO:20)

* sequence represents cDNA sequence of MBP up to the restriction site in the polylinker where cDNA encoding SK was inserted.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: President and Fellows of Harvard College

(ii) TITLE OF INVENTION: PLASMIN-RESISTANT STREPTOKINASE

(iii) NUMBER OF SEQUENCES: 20

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US96/-----
(B) FILING DATE: 07-JUN-1996
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/488,940
(B) FILING DATE: 09-JUN-1995

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1194 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly Asp Lys
1 5 10 15

Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu Lys Asp Thr
20 25 30

Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu Lys Phe
35 40 45

Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe Trp Ala
50 55 60

His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala Glu Ile
65 70 75 80

Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe Thr Trp Asp
85 90 95

Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala Val Glu
100 105 110

Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro Pro Lys
115 120 125

Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala Lys Gly
130 135 140

Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro
145 150 155 160

Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys
165 170 175

Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly
180 185 190

Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp
195 200 205

Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala
210 215 220

Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys
225 230 235 240

Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser
245 250 255

Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro
260 265 270

Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp
275 280 285

Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala
290 295 300

Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala
305 310 315 320

Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln
325 330 335

Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala
340 345 350

Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn
355 360 365

Ser Ser Ser Val Pro Gly Arg Gly Ser Ile Glu Gly Arg Ile Ala Gly
370 375 380

Pro Glu Trp Leu Leu Asp Arg Pro Ser Val Asn Asn Ser Gln Leu Val
385 390 395 400

Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp Ile Ser Leu
405 410 415

Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg Pro Ala His Gly Gly Lys
420 425 430

Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys Pro Phe Ala Thr Asp Ser
435 440 445

Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu Lys Ala Ile
450 455 460

Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp Tyr Phe Glu
465 470 475 480

Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg Asn Gly Lys
485 490 495

Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro Thr Gln Pro
500 505 510

Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg Tyr Lys Glu
515 520 525

Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu Tyr Thr Val
530 535 540

Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro Gly Leu Lys
545 550 555 560

Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr Ile Thr Ser
565 570 575

Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys Asn His Pro
580 585 590

Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr His Asp Asn
595 600 605

Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe Thr Tyr Arg
610 615 620

Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys Ser Gly Leu
625 630 635 640

Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys Tyr Tyr Val
645 650 655

Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg Ser His Leu
660 665 670

Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn Glu Leu Leu
675 680 685

Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu Asp Phe Arg
690 695 700

Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr Asn Asn Leu
705 710 715 720

Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys Val Glu Asp
725 730 735

Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met Gly Lys Arg
740 745 750

Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp Lys Asp Arg
755 760 765

Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg Tyr Thr Gly
770 775 780

Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys Asn Asn Ser Gln Leu Val
785 790 795 800

Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp Ile Ser Leu
805 810 815

Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg Pro Ala His Gly Gly Lys
820 825 830

Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys Pro Phe Ala Thr Asp Ser
835 840 845

Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu Lys Ala Ile
850 855 860

Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp Tyr Phe Glu
865 870 875 880

Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg Asn Gly Lys
885 890 895

Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro Thr Gln Pro
900 905 910

Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg Tyr Lys Glu
915 920 925

Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu Tyr Thr Val
930 935 940

Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro Gly Leu Lys
945 950 955 960

Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr Ile Thr Ser
965 970 975

Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys Asn His Pro
980 985 990

Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr His Asp Asn
995 1000 1005

Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe Thr Tyr Arg
1010 1015 1020

Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys Ser Gly Leu
1025 1030 1035 1040

Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys Tyr Tyr Val
1045 1050 1055

Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg Ser His Leu
1060 1065 1070

Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn Glu Leu Leu
1075 1080 1085

Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu Asp Phe Arg
1090 1095 1100

Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr Asn Asn Leu
1105 1110 1115 1120

Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys Val Glu Asp
1125 1130 1135

Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met Gly Lys Arg
1140 1145 1150

Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp Lys Asp Arg
1155 1160 1165

Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg Tyr Thr Gly
1170 1175 1180

Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys
1185 1190

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1181 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly Asp Lys
1 5 10 15

Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu Lys Asp Thr
20 25 30

Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu Lys Phe
35 40 45

Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe Trp Ala
50 55 60

His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala Glu Ile
65 70 75 80

Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe Thr Trp Asp
85 90 95

Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala Val Glu
100 105 110

Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro Pro Lys
115 120 125

Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala Lys Gly
130 135 140

Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro
145 150 155 160

Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys
165 170 175

Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly
180 185 190

Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp
195 200 205

Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala
210 215 220

Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys
225 230 235 240

Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser
245 250 255

Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro
260 265 270

Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp
275 280 285

Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala
290 295 300

Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala
305 310 315 320

Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln
325 330 335

Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala
340 345 350

Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn
355 360 365

Ser Ser Ser Val Pro Gly Arg Gly Ser Ile Glu Gly Arg Asn Asn Ser
370 375 380

Gln Leu Val Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp
385 390 395 400

Ile Ser Leu Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg Pro Ala His
405 410 415

Gly Gly Lys Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys Pro Phe Ala
420 425 430

Thr Asp Ser Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu
435 440 445

Lys Ala Ile Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp
450 455 460

Tyr Phe Glu Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg
465 470 475 480

Asn Gly Lys Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro
485 490 495

Thr Gln Pro Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg
500 505 510

Tyr Lys Glu Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu
515 520 525

Tyr Thr Val Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro
530 535 540

Gly Leu Lys Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr
545 550 555 560

Ile Thr Ser Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys
565 570 575

Asn His Pro Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr
580 585 590

His Asp Asn Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe
595 600 605

Thr Tyr Arg Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys
610 615 620

Ser Gly Leu Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys
625 630 635 640

Tyr Tyr Val Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg
645 650 655

Ser His Leu Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn
660 665 670

Glu Leu Leu Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu
675 680 685

Asp Phe Arg Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr
690 695 700

Asn Asn Leu Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys
705 710 715 720

Val Glu Asp Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met
725 730 735

Gly Lys Arg Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp
740 745 750

Lys Asp Arg Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg
755 760 765

Tyr Thr Gly Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys Asn Asn Ser
770 775 780

Gln Leu Val Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp
785 790 795 800

Ile Ser Leu Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg Pro Ala His
805 810 815

Gly Gly Lys Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys Pro Phe Ala
820 825 830

Thr Asp Ser Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu
835 840 845

Lys Ala Ile Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp
850 855 860

Tyr Phe Glu Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg
865 870 875 880

Asn Gly Lys Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro
885 890 895

Thr Gln Pro Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg
900 905 910

Tyr Lys Glu Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu
915 920 925

Tyr Thr Val Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro
930 935 940

Gly Leu Lys Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr
945 950 955 960

Ile Thr Ser Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys
965 970 975

Asn His Pro Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr
980 985 990

His Asp Asn Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe
995 1000 1005

Thr Tyr Arg Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys
1010 1015 1020

Ser Gly Leu Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys
1025 1030 1035 1040

Tyr Tyr Val Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg
1045 1050 1055

Ser His Leu Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn
1060 1065 1070

Glu Leu Leu Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu
1075 1080 1085

Asp Phe Arg Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr
1090 1095 1100

Asn Asn Leu Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys
1105 1110 1115 1120

Val Glu Asp Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met
1125 1130 1135

Gly Lys Arg Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp
1140 1145 1150

Lys Asp Arg Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg
1155 1160 1165

Tyr Thr Gly Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys
1170 1175 1180

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 813 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ile Ala Gly Pro Glu Trp Leu Leu Asp Arg Pro Ser Val Asn Asn Ser
1 5 10 15

Gln Leu Val Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp
20 25 30

Ile Ser Leu Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg Pro Ala His
35 40 45

Gly Gly Lys Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys Pro Phe Ala
50 55 60

Thr Asp Ser Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu
65 70 75 80

Lys Ala Ile Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp
85 90 95

Tyr Phe Glu Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg
100 105 110

Asn Gly Lys Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro
115 120 125

Thr Gln Pro Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg
130 135 140

Tyr Lys Glu Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu
145 150 155 160

Tyr Thr Val Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro
165 170 175

Gly Leu Lys Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr
180 185 190

Ile Thr Ser Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys
195 200 205

Asn His Pro Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr
210 215 220

His Asp Asn Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe
225 230 235 240

Thr Tyr Arg Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys
245 250 255

Ser Gly Leu Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys
260 265 270

Tyr Tyr Val Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg
275 280 285

Ser His Leu Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn
290 295 300

Glu Leu Leu Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu
305 310 315 320

Asp Phe Arg Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr
325 330 335

Asn Asn Leu Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys
340 345 350

Val Glu Asp Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met
355 360 365

Gly Lys Arg Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp
370 375 380

Lys Asp Arg Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg
385 390 395 400

Tyr Thr Gly Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys Asn Asn Ser
405 410 415

Gln Leu Val Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp
420 425 430

Ile Ser Leu Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg Pro Ala His
435 440 445

Gly Gly Lys Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys Pro Phe Ala
450 455 460

Thr Asp Ser Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu
465 470 475 480

Lys Ala Ile Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp
485 490 495

Tyr Phe Glu Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg
500 505 510

Asn Gly Lys Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro
515 520 525

Thr Gln Pro Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg
530 535 540

Tyr Lys Glu Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu
545 550 555 560

Tyr Thr Val Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro
565 570 575

Gly Leu Lys Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr
580 585 590

Ile Thr Ser Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys
595 600 605

Asn His Pro Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr
610 615 620

His Asp Asn Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe
625 630 635 640

Thr Tyr Arg Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys
645 650 655

Ser Gly Leu Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys
660 665 670

Tyr Tyr Val Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg
675 680 685

Ser His Leu Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn
690 695 700

Glu Leu Leu Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu
705 710 715 720

Asp Phe Arg Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr
725 730 735

Asn Asn Leu Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys
740 745 750

Val Glu Asp Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met
755 760 765

Gly Lys Arg Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp
770 775 780

Lys Asp Arg Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg
785 790 795 800

Tyr Thr Gly Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys
805 810

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 800 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asn Asn Ser Gln Leu Val Val Ser Val Ala Gly Thr Val Glu Gly Thr
1 5 10 15

Asn Gln Asp Ile Ser Leu Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg
20 25 30

Pro Ala His Gly Gly Lys Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys
35 40 45

Pro Phe Ala Thr Asp Ser Gly Ala Met Ser His Lys Leu Glu Lys Ala
50 55 60

Asp Leu Leu Lys Ala Ile Gln Glu Gln Leu Ile Ala Asn Val His Ser
65 70 75 80

Asn Asp Asp Tyr Phe Glu Val Ile Asp Phe Ala Ser Asp Ala Thr Ile
85 90 95

Thr Asp Arg Asn Gly Lys Val Tyr Phe Ala Asp Lys Asp Gly Ser Val
100 105 110

Thr Leu Pro Thr Gln Pro Val Gln Glu Phe Leu Leu Ser Gly His Val
115 120 125

Arg Val Arg Tyr Lys Glu Lys Pro Ile Gln Asn Gln Ala Lys Ser Val
130 135 140

Asp Val Glu Tyr Thr Val Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp
145 150 155 160

Phe Arg Pro Gly Leu Lys Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile
165 170 175

Gly Asp Thr Ile Thr Ser Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile
180 185 190

Leu Asn Lys Asn His Pro Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser
195 200 205

Ile Val Thr His Asp Asn Asp Ile Phe Arg Thr Ile Leu Pro Met Asp
210 215 220

Gln Glu Phe Thr Tyr Arg Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile
225 230 235 240

Asn Lys Lys Ser Gly Leu Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile
245 250 255

Ser Glu Lys Tyr Tyr Val Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro
260 265 270

Phe Asp Arg Ser His Leu Lys Leu Phe Thr Ile Lys Tyr Val Asp Val
275 280 285

Asp Thr Asn Glu Leu Leu Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu
290 295 300

Arg Asn Leu Asp Phe Arg Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys
305 310 315 320

Leu Leu Tyr Asn Asn Leu Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu
325 330 335

Thr Gly Lys Val Glu Asp Asn His Asp Asp Thr Asn Arg Ile Ile Thr
340 345 350

Val Tyr Met Gly Lys Arg Pro Glu Gly Glu Asn Ala Ser Tyr His Leu
355 360 365

Ala Tyr Asp Lys Asp Arg Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser
370 375 380

Tyr Leu Arg Tyr Thr Gly Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys
385 390 395 400

Asn Asn Ser Gln Leu Val Val Ser Val Ala Gly Thr Val Glu Gly Thr
405 410 415

Asn Gln Asp Ile Ser Leu Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg
420 425 430

Pro Ala His Gly Gly Lys Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys
435 440 445

Pro Phe Ala Thr Asp Ser Gly Ala Met Ser His Lys Leu Glu Lys Ala
450 455 460

Asp Leu Leu Lys Ala Ile Gln Glu Gln Leu Ile Ala Asn Val His Ser
465 470 475 480

Asn Asp Asp Tyr Phe Glu Val Ile Asp Phe Ala Ser Asp Ala Thr Ile
485 490 495

Thr Asp Arg Asn Gly Lys Val Tyr Phe Ala Asp Lys Asp Gly Ser Val
500 505 510

Thr Leu Pro Thr Gln Pro Val Gln Glu Phe Leu Leu Ser Gly His Val
515 520 525

Arg Val Arg Tyr Lys Glu Lys Pro Ile Gln Asn Gln Ala Lys Ser Val
530 535 540

Asp Val Glu Tyr Thr Val Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp
545 550 555 560

Phe Arg Pro Gly Leu Lys Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile
565 570 575

Gly Asp Thr Ile Thr Ser Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile
580 585 590

Leu Asn Lys Asn His Pro Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser
595 600 605

Ile Val Thr His Asp Asn Asp Ile Phe Arg Thr Ile Leu Pro Met Asp
610 615 620

Gln Glu Phe Thr Tyr Arg Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile
625 630 635 640

Asn Lys Lys Ser Gly Leu Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile
645 650 655

Ser Glu Lys Tyr Tyr Val Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro
660 665 670

Phe Asp Arg Ser His Leu Lys Leu Phe Thr Ile Lys Tyr Val Asp Val
675 680 685

Asp Thr Asn Glu Leu Leu Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu
690 695 700

Arg Asn Leu Asp Phe Arg Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys
705 710 715 720

Leu Leu Tyr Asn Asn Leu Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu
725 730 735

Thr Gly Lys Val Glu Asp Asn His Asp Asp Thr Asn Arg Ile Ile Thr
740 745 750

Val Tyr Met Gly Lys Arg Pro Glu Gly Glu Asn Ala Ser Tyr His Leu
755 760 765

Ala Tyr Asp Lys Asp Arg Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser
770 775 780

Tyr Leu Arg Tyr Thr Gly Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys
785 790 795 800

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GCTGCTAGAC GCGCCATCTG TCAAC 25

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TGGCGCGTCT AGCAGCCACT CAG 23

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAAGACATTA GTCTGGCCTT TTTTGAAATC G 31

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGCCAGACTA ATGTCTTGAT TCG 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGATCTAACA TCGGCGCCTG CTCATGG 27

(2) INFORMATION FOR SEQ ID NO:10:

CGATCTAACA TCGGCGCCTG CTCATGG 27

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGCCGATGTT AGATCGATTT C 21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCTCATGGAG GCGCCACAGA GGGC 24

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GGCGCCTCCA TGAGCAGGTC 20

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCTTAAGTCC GGCCTCAAAA CCATTTGC 28

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGAGGCCGGA CTTAAGCCTT GCTC 24

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GCCGATCGAT ATACCGAAGA AGAACGAG 28

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TATCGATCGG CATCATAGGC TAAATGATAG C 31

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1194 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Lys Thr Glu Glu Gly Lys Leu Val Ile Trp Ile Asn Gly Asp Lys
1 5 10 15

Gly Tyr Asn Gly Leu Ala Glu Val Gly Lys Lys Phe Glu Lys Asp Thr
20 25 30

Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu Lys Phe
35 40 45

Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe Trp Ala
50 55 60

His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala Glu Ile
65 70 75 80

Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe Thr Trp Asp
85 90 95

Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala Val Glu
100 105 110

Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro Pro Lys
115 120 125

Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala Lys Gly
130 135 140

Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro
145 150 155 160

Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys
165 170 175

Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly
180 185 190

Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp
195 200 205

Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala
210 215 220

Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys
225 230 235 240

Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser
245 250 255

Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro
260 265 270

Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp
275 280 285

Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala
290 295 300

Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala
305 310 315 320

Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln
325 330 335

Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala
340 345 350

Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn
355 360 365

Ser Ser Ser Val Pro Gly Arg Gly Ser Ile Glu Gly Arg Ile Ala Gly
370 375 380

Pro Glu Trp Leu Leu Asp Ala Pro Ser Val Asn Asn Ser Gln Leu Val
385 390 395 400

Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp Ile Ser Leu
405 410 415

Ala Phe Phe Glu Ile Asp Leu Thr Ser Ala Pro Ala His Gly Gly Ala
420 425 430

Thr Glu Gln Gly Leu Ser Pro Ala Ser Lys Pro Phe Ala Thr Asp Ser
435 440 445

Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu Lys Ala Ile
450 455 460

Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp Tyr Phe Glu
465 470 475 480

Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg Asn Gly Lys
485 490 495

Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro Thr Gln Pro
500 505 510

Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg Tyr Lys Glu
515 520 525

Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu Tyr Thr Val
530 535 540

Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro Gly Leu Lys
545 550 555 560

Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr Ile Thr Ser
565 570 575

Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys Asn His Pro
580 585 590

Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr His Asp Asn
595 600 605

Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe Thr Tyr Arg
610 615 620

Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys Ser Gly Leu
625 630 635 640

Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys Tyr Tyr Val
645 650 655

Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg Ser His Leu
660 665 670

Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn Glu Leu Leu
675 680 685

Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu Asp Phe Arg
690 695 700

Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr Asn Asn Leu
705 710 715 720

Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys Val Glu Asp
725 730 735

Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met Gly Lys Arg
740 745 750

Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp Lys Asp Arg
755 760 765

Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg Tyr Thr Gly
770 775 780

Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys Asn Asn Ser Gln Leu Val
785 790 795 800

Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp Ile Ser Leu
805 810 815

Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg Pro Ala His Gly Gly Lys
820 825 830

Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys Pro Phe Ala Thr Asp Ser
835 840 845

Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu Lys Ala Ile
850 855 860

Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp Tyr Phe Glu
865 870 875 880

Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg Asn Gly Lys
885 890 895

Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro Thr Gln Pro
900 905 910

Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg Tyr Lys Glu
915 920 925

Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu Tyr Thr Val
930 935 940

Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro Gly Leu Lys
945 950 955 960

```
Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr Ile Thr Ser
                965                 970                 975

Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys Asn His Pro
            980                 985                 990

Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr His Asp Asn
        995                 1000                1005

Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe Thr Tyr Arg
    1010                1015                1020

Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys Ser Gly Leu
1025                1030                1035                1040

Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys Tyr Tyr Val
                1045                1050                1055

Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg Ser His Leu
            1060                1065                1070

Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn Glu Leu Leu
        1075                1080                1085

Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu Asp Phe Arg
    1090                1095                1100

Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr Asn Asn Leu
1105                1110                1115                1120

Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys Val Glu Asp
                1125                1130                1135

Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met Gly Lys Arg
            1140                1145                1150

Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp Lys Asp Arg
        1155                1160                1165

Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg Tyr Thr Gly
    1170                1175                1180

Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys
1185                1190
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1194 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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1               5                  10                  15
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20 25 30

Gly Ile Lys Val Thr Val Glu His Pro Asp Lys Leu Glu Glu Lys Phe
35 40 45

Pro Gln Val Ala Ala Thr Gly Asp Gly Pro Asp Ile Ile Phe Trp Ala
50 55 60

His Asp Arg Phe Gly Gly Tyr Ala Gln Ser Gly Leu Leu Ala Glu Ile
65 70 75 80

Thr Pro Asp Lys Ala Phe Gln Asp Lys Leu Tyr Pro Phe Thr Trp Asp
85 90 95

Ala Val Arg Tyr Asn Gly Lys Leu Ile Ala Tyr Pro Ile Ala Val Glu
100 105 110

Ala Leu Ser Leu Ile Tyr Asn Lys Asp Leu Leu Pro Asn Pro Pro Lys
115 120 125

Thr Trp Glu Glu Ile Pro Ala Leu Asp Lys Glu Leu Lys Ala Lys Gly
130 135 140

Lys Ser Ala Leu Met Phe Asn Leu Gln Glu Pro Tyr Phe Thr Trp Pro
145 150 155 160

Leu Ile Ala Ala Asp Gly Gly Tyr Ala Phe Lys Tyr Glu Asn Gly Lys
165 170 175

Tyr Asp Ile Lys Asp Val Gly Val Asp Asn Ala Gly Ala Lys Ala Gly
180 185 190

Leu Thr Phe Leu Val Asp Leu Ile Lys Asn Lys His Met Asn Ala Asp
195 200 205

Thr Asp Tyr Ser Ile Ala Glu Ala Ala Phe Asn Lys Gly Glu Thr Ala
210 215 220

Met Thr Ile Asn Gly Pro Trp Ala Trp Ser Asn Ile Asp Thr Ser Lys
225 230 235 240

Val Asn Tyr Gly Val Thr Val Leu Pro Thr Phe Lys Gly Gln Pro Ser
245 250 255

Lys Pro Phe Val Gly Val Leu Ser Ala Gly Ile Asn Ala Ala Ser Pro
260 265 270

Asn Lys Glu Leu Ala Lys Glu Phe Leu Glu Asn Tyr Leu Leu Thr Asp
275 280 285

Glu Gly Leu Glu Ala Val Asn Lys Asp Lys Pro Leu Gly Ala Val Ala
290 295 300

Leu Lys Ser Tyr Glu Glu Glu Leu Ala Lys Asp Pro Arg Ile Ala Ala
305 310 315 320

Thr Met Glu Asn Ala Gln Lys Gly Glu Ile Met Pro Asn Ile Pro Gln
325 330 335

Met Ser Ala Phe Trp Tyr Ala Val Arg Thr Ala Val Ile Asn Ala Ala
340 345 350

Ser Gly Arg Gln Thr Val Asp Glu Ala Leu Lys Asp Ala Gln Thr Asn
355 360 365

Ser Ser Ser Val Pro Gly Arg Gly Ser Ile Glu Gly Arg Ile Ala Gly
370 375 380

Pro Glu Trp Leu Leu Asp Ala Pro Ser Val Asn Asn Ser Gln Leu Val
385 390 395 400

Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp Ile Ser Leu
405 410 415

Ala Phe Phe Glu Ile Asp Leu Thr Ser Ala Pro Ala His Gly Gly Ala
420 425 430

Thr Glu Gln Gly Leu Ser Pro Ala Ser Lys Pro Phe Ala Thr Asp Ser
435 440 445

Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu Lys Ala Ile
450 455 460

Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp Tyr Phe Glu
465 470 475 480

Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg Asn Gly Lys
485 490 495

Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro Thr Gln Pro
500 505 510

Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg Tyr Lys Glu
515 520 525

Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu Tyr Thr Val
530 535 540

Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro Gly Leu Lys
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Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr Ile Thr Ser
565 570 575

Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys Asn His Pro
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Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr His Asp Asn
595 600 605

Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe Thr Tyr Arg
610 615 620

Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys Ser Gly Leu
625 630 635 640

Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys Tyr Tyr Val
645 650 655

Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg Ser His Leu
660 665 670

Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn Glu Leu Leu
675 680 685

Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu Asp Phe Arg
690 695 700

Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr Asn Asn Leu
705 710 715 720

Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys Val Glu Asp
725 730 735

Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met Gly Lys Arg
740 745 750

Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp Ala Asp Arg
755 760 765

Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg Tyr Thr Gly
770 775 780

Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys Asn Asn Ser Gln Leu Val
785 790 795 800

Val Ser Val Ala Gly Thr Val Glu Gly Thr Asn Gln Asp Ile Ser Leu
805 810 815

Lys Phe Phe Glu Ile Asp Leu Thr Ser Arg Pro Ala His Gly Gly Lys
820 825 830

Thr Glu Gln Gly Leu Ser Pro Lys Ser Lys Pro Phe Ala Thr Asp Ser
835 840 845

Gly Ala Met Ser His Lys Leu Glu Lys Ala Asp Leu Leu Lys Ala Ile
850 855 860

Gln Glu Gln Leu Ile Ala Asn Val His Ser Asn Asp Asp Tyr Phe Glu
865 870 875 880

Val Ile Asp Phe Ala Ser Asp Ala Thr Ile Thr Asp Arg Asn Gly Lys
885 890 895

Val Tyr Phe Ala Asp Lys Asp Gly Ser Val Thr Leu Pro Thr Gln Pro
900 905 910

Val Gln Glu Phe Leu Leu Ser Gly His Val Arg Val Arg Tyr Lys Glu
915 920 925

Lys Pro Ile Gln Asn Gln Ala Lys Ser Val Asp Val Glu Tyr Thr Val
930 935 940

Gln Phe Thr Pro Leu Asn Pro Asp Asp Asp Phe Arg Pro Gly Leu Lys
945 950 955 960

Asp Thr Lys Leu Leu Lys Thr Leu Ala Ile Gly Asp Thr Ile Thr Ser
965 970 975

Gln Glu Leu Leu Ala Gln Ala Gln Ser Ile Leu Asn Lys Asn His Pro
980 985 990

Gly Tyr Thr Ile Tyr Glu Arg Asp Ser Ser Ile Val Thr His Asp Asn
995 1000 1005

Asp Ile Phe Arg Thr Ile Leu Pro Met Asp Gln Glu Phe Thr Tyr Arg
1010 1015 1020

Val Lys Asn Arg Glu Gln Ala Tyr Arg Ile Asn Lys Lys Ser Gly Leu
1025 1030 1035 1040

Asn Glu Glu Ile Asn Asn Thr Asp Leu Ile Ser Glu Lys Tyr Tyr Val
1045 1050 1055

Leu Lys Lys Gly Glu Lys Pro Tyr Asp Pro Phe Asp Arg Ser His Leu
1060 1065 1070

Lys Leu Phe Thr Ile Lys Tyr Val Asp Val Asp Thr Asn Glu Leu Leu
1075 1080 1085

Lys Ser Glu Gln Leu Leu Thr Ala Ser Glu Arg Asn Leu Asp Phe Arg
1090 1095 1100

Asp Leu Tyr Asp Pro Arg Asp Lys Ala Lys Leu Leu Tyr Asn Asn Leu
1105 1110 1115 1120

Asp Ala Phe Gly Ile Met Asp Tyr Thr Leu Thr Gly Lys Val Glu Asp
1125 1130 1135

Asn His Asp Asp Thr Asn Arg Ile Ile Thr Val Tyr Met Gly Lys Arg
1140 1145 1150

Pro Glu Gly Glu Asn Ala Ser Tyr His Leu Ala Tyr Asp Lys Asp Arg
1155 1160 1165

Tyr Thr Glu Glu Glu Arg Glu Val Tyr Ser Tyr Leu Arg Tyr Thr Gly
1170 1175 1180

Thr Pro Ile Pro Asp Asn Pro Asn Asp Lys
1185 1190

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2566 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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GACATAAAAA TGCTGTTTGG GTTGTGCTGA TAGGCAAAAT GACCTCAAGC CCTGCAATCA 180

TCTGCTGGAG CAACTCAACT AAGTCAGCTG GTAAAACCTG CTGATGATTG AGGTAAATAA 240

ACTGAGAAGT CTCAAACAGC TGAGGGGGAT TGCCCTGATG ATCAAGCAAA TACCGCTGCC 300

AAGGTGACCC TAGCGGCTGC AAGACCTCAT ATTGACCCAA CCCCACCTCA AGTAATAAGC 360

GCTCTTTTTC GGATAAACAT GATTTGGGAA AATGCACATA TTGGTCCCCT TCTTTGACAC 420

TCACCCACTC TTTATCTCCT AACGGATGAG GGCCTACTTG CATCTCTGGA AAATAGTCTT 480

TTAGCTCCAT AGCCATTCCT TTCATGACGG TCTTTAAACC ATTATAACAC ATGACTCTTT 540

ATCACACAGT TCAGTTTGTT GTCAGCACGA TTTTGTATTT TCTGCCTTTT TAATCATTAA 600

AACTAAATAA GGGTTATTCA TTTTTAGCAA GAACATTCAA TTAAATAGCT ATTTATCGGA 660

ATATTAATTT ATGTTTATGC TAAAAAAGGT ATTATTTACC TTTTTTCATT GTCATTAAAA 720

TATCATTTTA AAAAAATCAA TAGGTTTTTA TTTGTGTCTT TAAAACCATT ATGTTATTCT 780

AATAATGGGG ATTGAAACTT AACTTTTAGG AGGTTTCTAT GAAAAATTAC TTATCTTTTG 840

GGATGTTTGC ACTGCTGTTT GCACTAACAT TTGGAACAGT CAATTCTGTC CAAGCTATTG 900

CTGGACCTGA GTGGCTGCTA GACCGTCCAT CTGTCAACAA CAGCCAATTA GTTGTTAGCG 960

TTGCTGGTAC TGTTGAGGGG ACGAATCAAG ACATTAGTCT TAAATTTTTT GAAATCGATC 1020

TAACATCACG ACCTGCTCAT AGGAAAGACA GAGCAAGGCT TAAGTCCAAA ATCAAAACCA 1080

TTTGCTACTG ATAGTGGCGC GATGTCACAT AAACTTGAGA AAGCTGACTT ACTAAAGGCT 1140

ATTCAAGAAC AATTGATCGC TAACGTCCAC AGTAACGACG ACTACTTTGA GGTCATTGAT 1200

TTTGCAAGCG ATGCAACCAT TACTGATCGA AACGGCAAGG TCTACTTTGC TGACAAAGAT 1260

GGTTCGGTAA CCTTGCCGAC CCAACCTGTC CAAGAATTTT TGCTAAGCGG ACATGTGCGC 1320

GTTAGACCAT ATAAAGAAAA ACCAATACAA AACCAAGCGA AATCTGTTGA TGTGGAATAT 1380

ACTGTACAGT TTACTCCCTT AAACCCTGAT GACGATTTCA GACCAGGTCT CAAAGATACT 1440

AAGCTATTGA AAACACTAGC TATCGGTGAC ACCATCACAT CTCAAGAATT ACTAGCTCAA 1500

GCACAAAGCA TTTTAAACAA AAACCACCCA GGCTATACGA TTTATGAACG TGACTCCTCA 1560

ATCGTCACTC ATGACAATGA CATTTTCCGT ACGATTTTAC CAATGGATCA AGAGTTTACT 1620

TACCGTGTTA AAAATCGGGA ACAAGCTTAT AGGATCAATA AAAAATCTGG TCTGAATGAA 1680

GAAATAAACA ACACTGACCT GATCTCTGAG AAATATTACG TCCTTAAAAA AGGGGAAAAG 1740

CCGTATGATC CCTTTGATCG CAGTCACTTG AAACTGTTCA CCATCAAATA CGTTGATGTC 1800

GATACCAACG AATTGCTAAA AAGTGAGCAG CTCTTAACAG CTAGCGAACG TAACTTAGAC 1860

TTCAGAGATT TATACGATCC TCGTGATAAG GCTAAACTAC TCTACAACAA TCTCGATGCT 1920

TTTGGTATTA TGGACTATAC CTTAACTGGA AAAGTAGAGG ATAATCACGA TGACACCAAC 1980

CGTATCATAA CCGTTTATAT GGGCAAGCGA CCCGAAGGAG AGAATGCTAG CTATCATTTA 2040

GCCTATGATA AAGATCGTTA TACCGAAGAA GAACGAGAAG TTTACAGCTA CCTGCGTTAT 2100

ACAGGGACAC CTATACCTGA TAACCCTAAC GACAAATAAC CACGGTCTTC TAAAACGATG 2160

AGATTAACTG ACAAAAAAAG CAAGCAACAT GCTATCAACA GTTGCTTGCT TTTTTCTAAC 2220

CTCTTAGTTG TAGAGACTAG TGACATTTCG TGTCTAAAAT AATCGTAACT GGTCCATCAT 2280

TGATGAGACT AACCTGCATA TCTGCCCCAA AAACGCCACG CTCAACTGGC ACAAAATCTG 2340

CCAATTGTTC ATTAAAGCGA TCATAAAACT GGCTAGCCAT ATCAGCTTTG CAGCTCCTGT 2400

AAAGGCTGGG CGATTTCCCT TTTTGGTGTC AGCATAAAGG GTAAATTGCG ACACAGATAA 2460

GATACTACCC TTGATGTCTT GGATAGACTG ATTCATCTTG CCATCAGCAT CTGAAAAAAT 2520

GCGCATGTTG ACTATTTTTG CACAGCGTAA GCCAAATCTT CTGCAG 2566

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1143 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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CTCGCTGAAG TCGGTAAGAA ATTCGAGAAA GATACCGGAA TTAAAGTCAC CGTTGAGCAT 120

CCGGATAAAC TGGAAGAGAA ATTCCCACAG GTTGCGGCAA CTGGCGATGG CCCTGACATT 180

ATCTTCTGGG CACACGACCG CTTTGGTGGC TACGCTCAAT CTGGCCTGTT GGCTGAAATC 240

ACCCCGGACA AAGCGTTCCA GGACAAGCTG TATCCGTTTA CCTGGGATGC CGTACGTTAC 300

AACGGCAAGC TGATTGCTTA CCCGATCGCT GTTGAAGCGT TATCGCTGAT TTATAACAAA 360

GATCTGCTGC CGAACCCGCC AAAAACCTGG GAAGAGATCC CGGCGCTGGA TAAAGAACTG 420

AAAGCGAAAG GTAAGAGCGC GCTGATGTTC AACCTGCAAG AACCGTACTT CACCTGGCCG 480

CTGATTGCTG CTGACGGGGG TTATGCGTTC AAGTATGAAA ACGGCAAGTA CGACATTAAA 540

GACGTGGGCG TGGATAACGC TGGCGCGAAA GCGGGTCTGA CCTTCCTGGT TGACCTGATT 600

AAAAACAAAC ACATGAATGC AGACACCGAT TACTCCATCG CAGAAGCTGC CTTTAATAAA 660

GGCGAAACAG CGATGACCAT CAACGGCCCG TGGGCATGGT CCAACATCGA CACCAGCAAA 720

GTGAATTATG GTGTAACGGT ACTGCCGACC TTCAAGGGTC AACCATCCAA ACCGTTCGTT 780

GGCGTGCTGA GCGCAGGTAT TAACGCCGCC AGTCCGAACA AAGAGCTGGC GAAAGAGTTC 840

CTCGAAAACT ATCTGCTGAC TGATGAAGGT CTGGAAGCGG TTAATAAAGA CAAACCGCTG 900

GGTGCCGTAG CGCTGAAGTC TTACGAGGAA GAGTTGGCGA AAGATCCACG TATTGCCGCC 960

ACCATGGAAA ACGCCCAGAA AGGTGAAATC ATGCCGAACA TCCCGCAGAT GTCCGCTTTC 1020

TGGTATGCCG TGCGTACTGC GGTGATCAAC GCCGCCAGCG GTCGTCAGAC TGTCGATGAA 1080

GCCCTGAAAG ACGCGCAGAC TAATTCGAGC TCGGTACCCG GCCGGGGATC CATCGAGGGT 1140

AGG 1143

Other embodiments are within the following claims:

What is claimed is:

1. A compound comprising (a) a plasminogen-binding fragment of streptokinase and (b) a blocking group at the amino-terminus of said fragment, wherein

(i) said compound is catalytically active; and

(ii) the rate of *in vitro* degradation of said compound in the presence of human plasminogen is at least 2 times slower compared to said rate of native streptokinase, wherein said rate is measured by the appearance of plasmin cleavage products as detected by immunoblotting using anti-streptokinase antibodies.

2. The compound of claim 1, wherein said compound comprises the amino acid sequence of SEQ ID NO: 4.

3. The compound of claim 1, wherein said blocking group is a heterologous peptide.

4. The compound of claim 3, wherein said heterologous peptide comprises at least one heterologous amino acid.

5. The compound of claim 4, wherein said heterologous peptide is maltose binding protein.

6. A DNA comprising a coding sequence encoding the compound of claim 3.

7. A method of dissolving blood clots in a mammal, comprising administering to said mammal an effective amount of the compound of claim 1.

8. A plasminogen-binding fragment of streptokinase, wherein

(a) said fragment lacks between 1 and 24 amino-terminal amino acids;

(b) said fragment is catalytically active; and

(c) the rate of *in vitro* degradation of said fragment in the presence of human plasminogen is at least 2 times slower compared to said rate of native streptokinase, wherein said rate is measured by the appearance of plasmin cleavage products as detected by immunoblotting using anti-streptokinase antibodies.

9. The fragment of claim 8, wherein said fragment comprises at least one mutation in a potential plasmin cleavage site, wherein said mutation renders said cleavage site resistant to cleavage by plasmin.

10. The fragment of claim 8, wherein said fragment consists of the amino acid sequence of (SEQ ID NO:4).

11. A DNA comprising a coding sequence encoding the fragment of claim 10.

12. A polypeptide comprising a plasminogen-binding fragment of streptokinase, wherein

(a) said fragment is catalytically active; and

(c) the rate of *in vitro* degradation of said polypeptide is at least two times slower compared to said rate of native streptokinase, wherein said rate is measured by the appearance of plasmin cleavage products as detected by immunoblotting using anti-streptokinase antibodies.

13. The polypeptide of claim 12, wherein said polypeptide comprises at least one mutation in a potential plasmin cleavage site, wherein said mutation renders said cleavage site resistant to cleavage by plasmin.

14. The polypeptide of claim 13, wherein said mutation is selected from the group consisting of R10A, K36A, R45A, K51A, K59A, K61A, K147A, K333, R232A, K257A, K298A, K309A, R234A, R363A, K386A, K372A, R388A, R394A, and R401A.

15. The polypeptide of claim 14, wherein said polypeptide comprises R10A, K36A, R45A, K51A and K59A (SEQ ID NO:17).

16. The polypeptide of claim 14, wherein said polypeptide comprises R10A, K36A, R45A, K51A, K59A and K386A (SEQ ID NO:18).

17. A DNA comprising a coding sequence encoding the polypeptide of claim 14.

18. A DNA comprising a coding sequence encoding the polypeptide of claim 15.

19. A method of dissolving blood clots in a mammal, comprising administering to said mammal an effective amount of the polypeptide of claim 15.

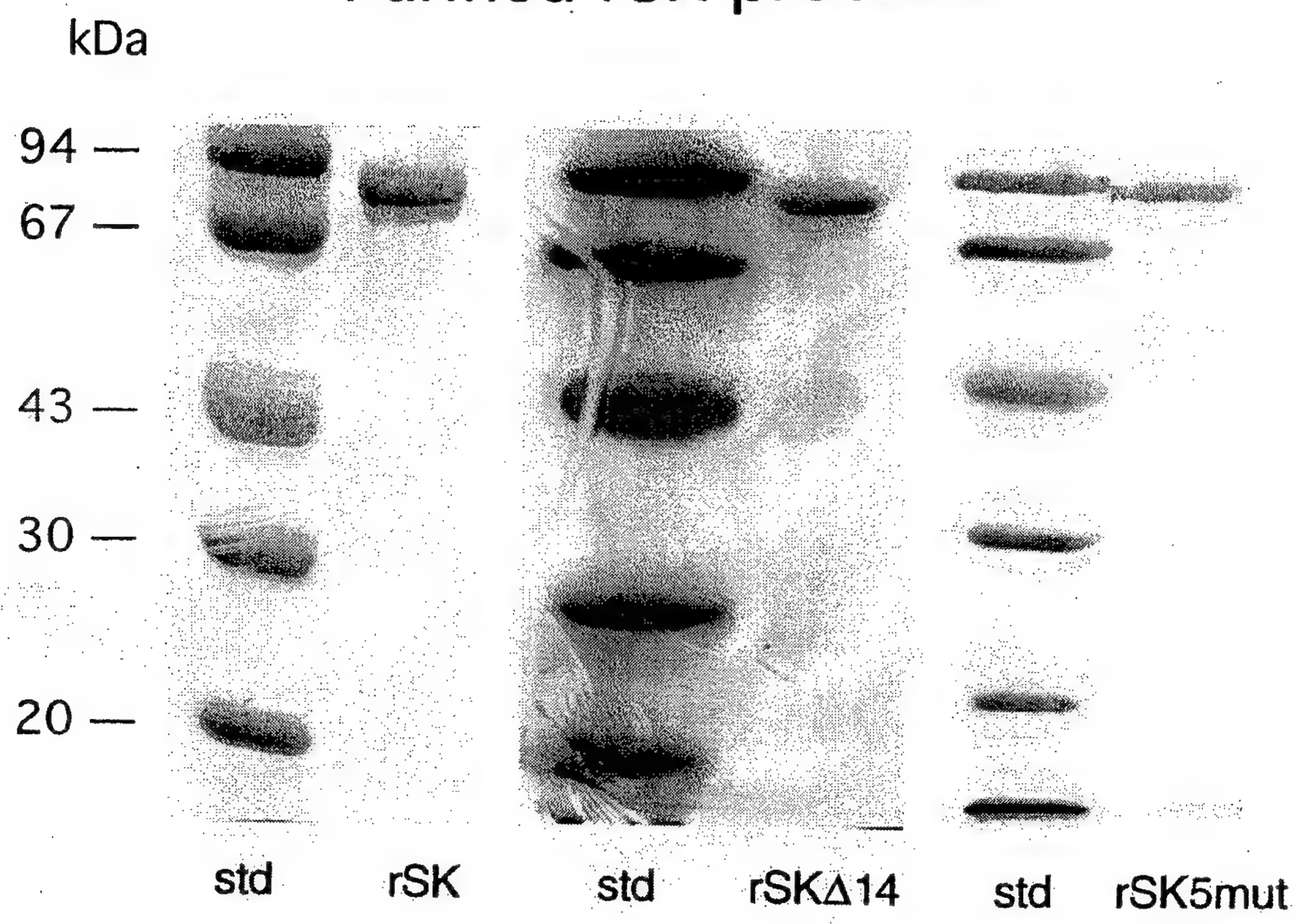

Purified rSK proteins
kDa
94
67
43
30
20
std
rSK
std
rSKΔ14
std
rSK5mut

FIG. 1

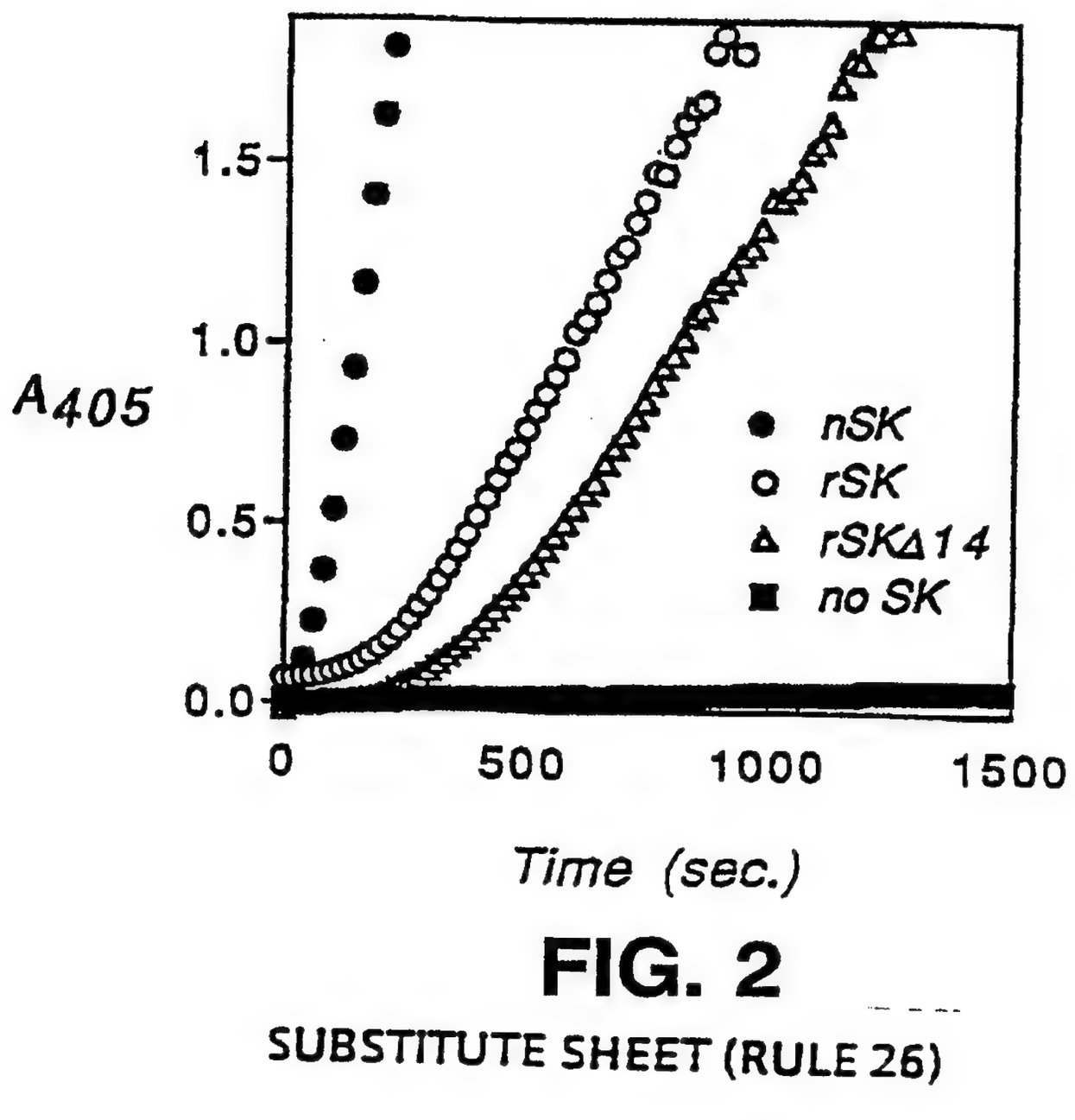

A405
1.5
1.0
0.5
0.0
0
500
1000
1500
Time (sec.)
nSK
rSK
rSKΔ14
no SK

FIG. 2

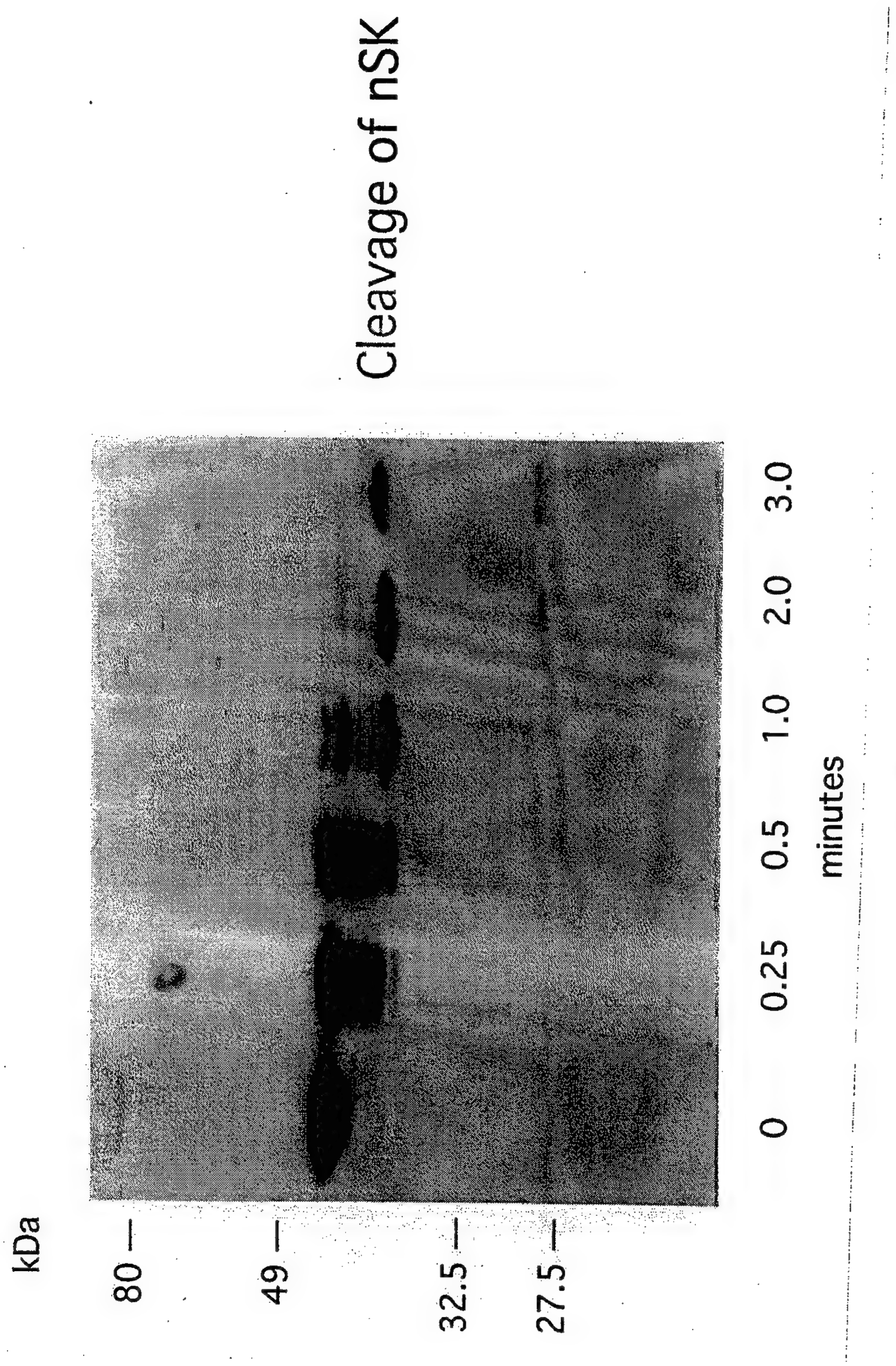


FIG. 3

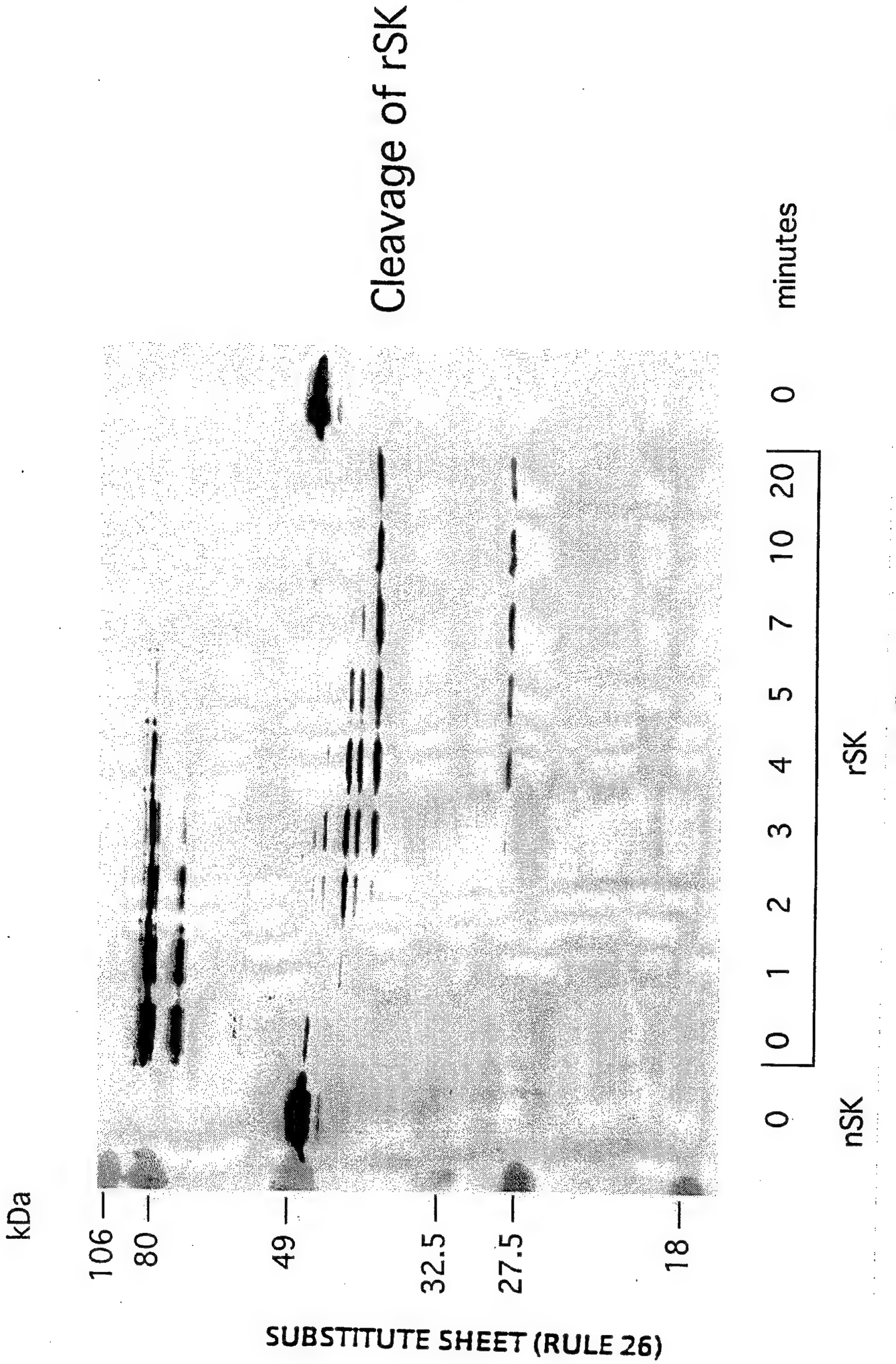

Cleavage of rSK
minutes
0
2
3
4
5
7
10
20
rSK
kDa
106
80
49
32.5
27.5
18
0
0
1
nSK

FIG. 4

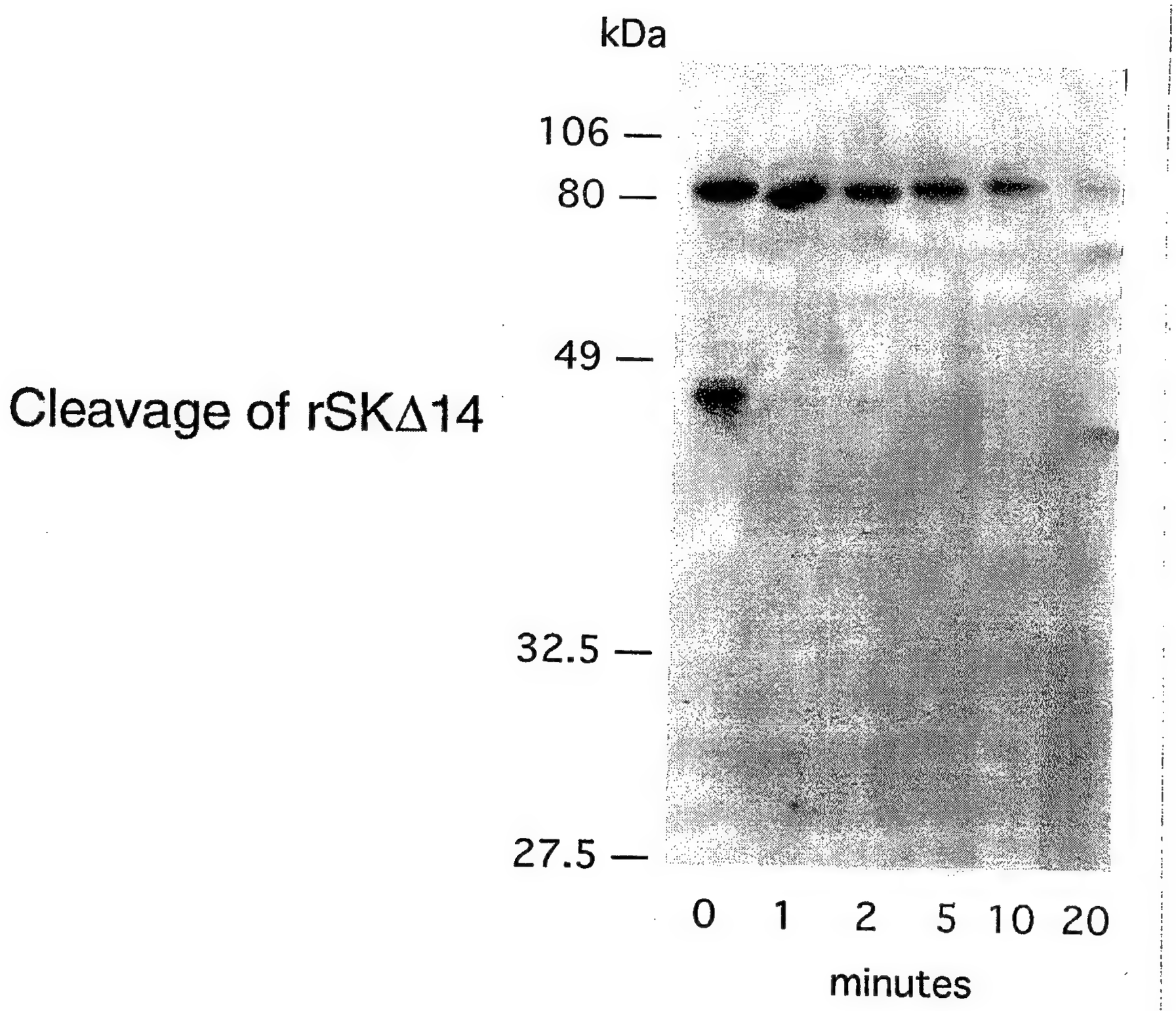
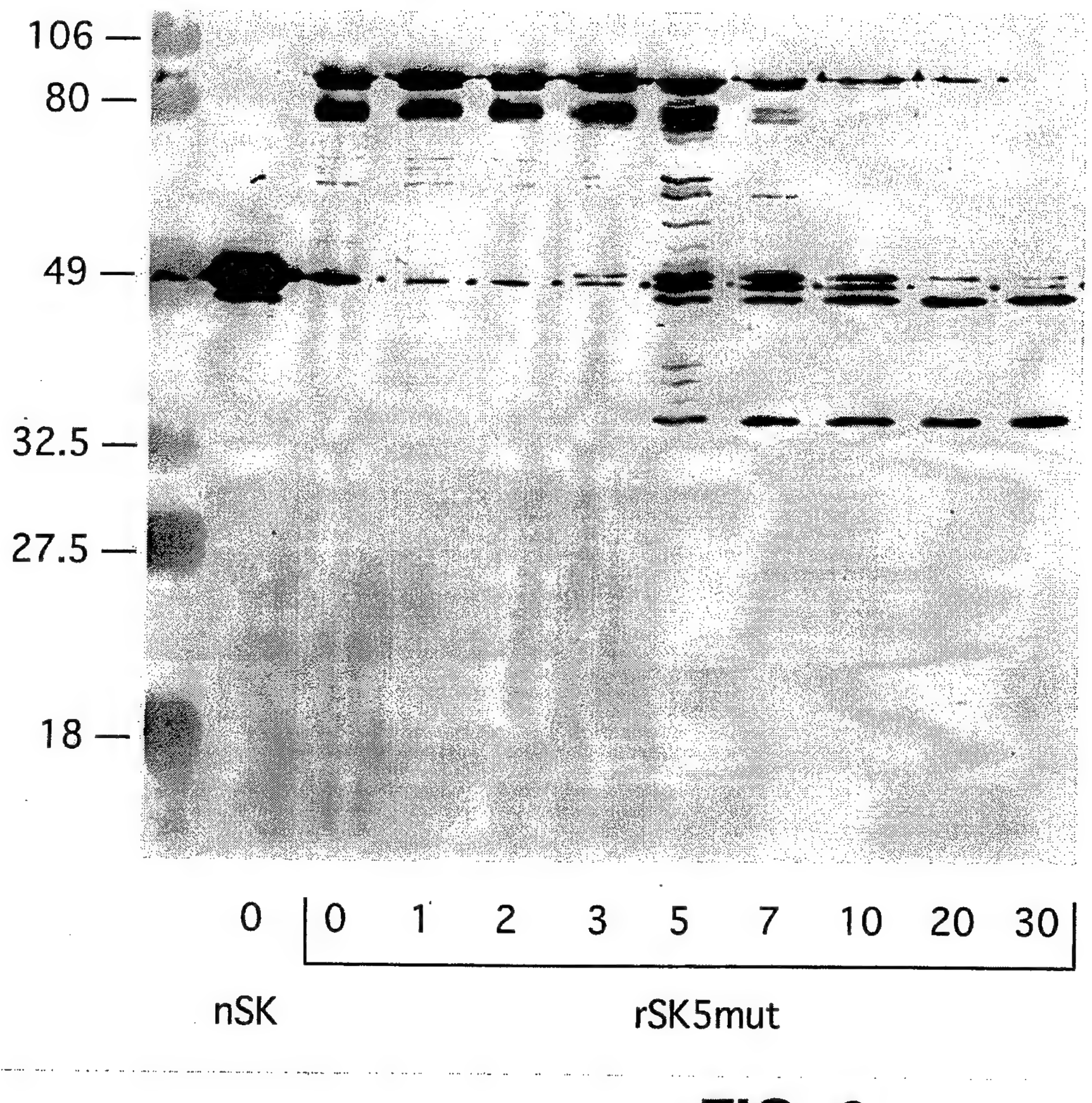


FIG. 5



Cleavage of rSK5mut

FIG. 6

Comparative cleavage of recombinant SKs

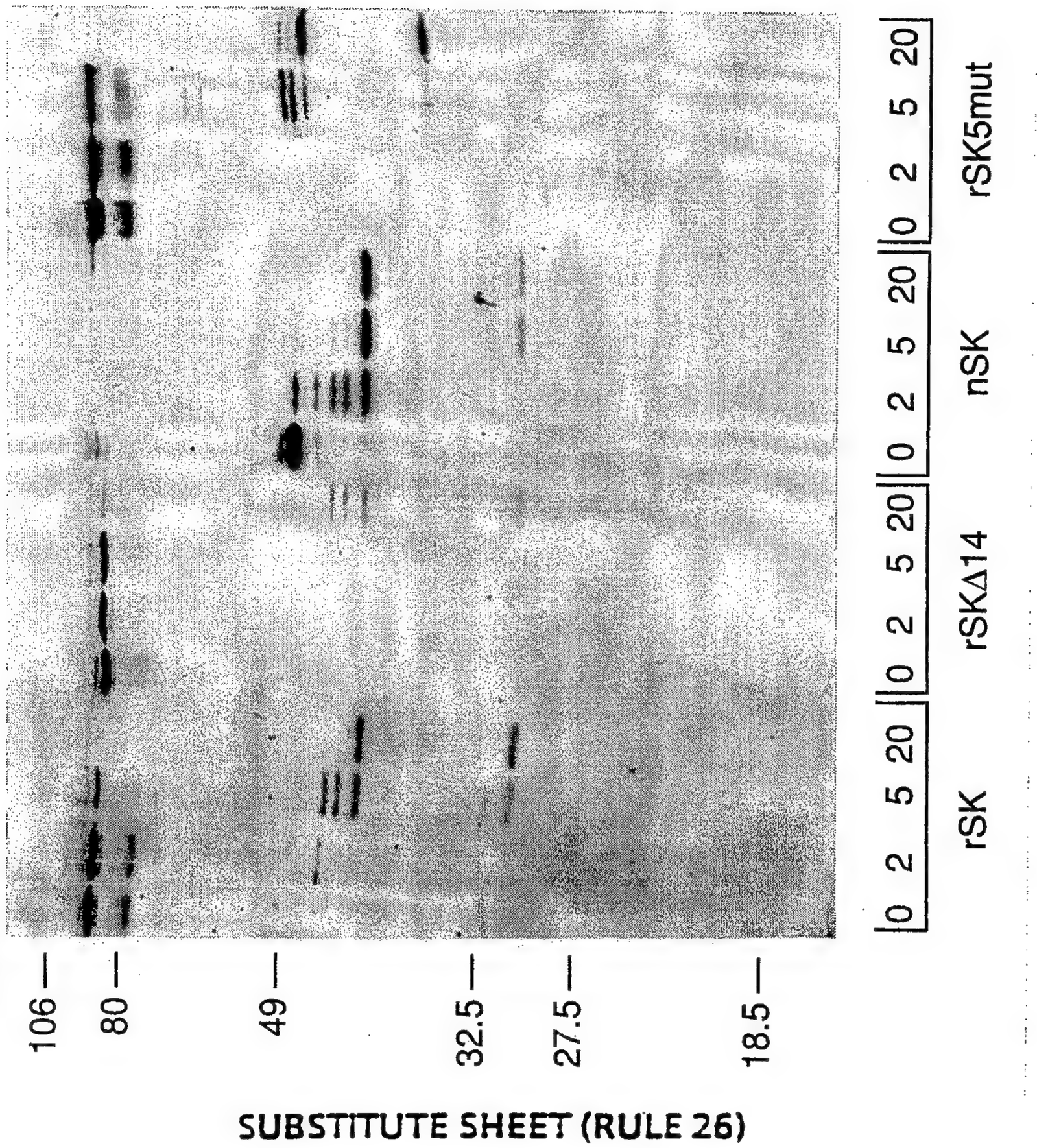


FIG. 7

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 96/09640

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/31 C07K14/315 A61K38/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO,A,94 07992 (GEN HOSPITAL CORP ;HARVARD COLLEGE (US)) 14 April 1994 see page 3, last paragraph see page 21, line 27 - page 24; example 2 ---	1-6,8, 10-12 9,13
X	MOLECULAR AND GENERAL GENETICS, vol. 212, 1988, pages 295-300, XP002016017 C. KLESSEN ET AL: "Tripartite streptokinase gene fusion vectors for gram-positive an gram-negative procaryotes" see the whole document --- -/--	1-4,6,8, 10-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 October 1996	04. 11. 96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Van der Schaal, C

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inte ional Application No
PC /US 96/09640

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BIOCHEMISTRY, vol. 29, 1990, pages 3585-3590, XP002016018 D. DAVIDSON ET AL: "Plasminogen activator activities of equimolar complexes of streptokinase with variant recombinant plasminogens" see the whole document	9,13
X	US,A,5 011 686 (PANG ROY H L) 30 April 1991 see page 3; claim 11	1,3,6,7
X	WO,A,91 09125 (BRITISH BIO TECHNOLOGY) 27 June 1991 see examples 8-10	1,3,6,7
A	JOURNAL OF CLINICAL INVESTIGATION, vol. 75, no. 2, 1985, pages 413-419, XP000605367 S. RAJAGOPALAN ET AL: "A nonantigenic covalent streptokinase-polyethylene glycol complex plasminogen activator function"	
P,X	68TH SCIENTIFIC SESSION OF THE AMERICAN HEART ASSOCIATION, ANAHEIM, CALIFORNIA, USA, NOVEMBER 13-16, 1995. CIRCULATION 92 (8 SUPPL.). 1995. I623. ISSN: 0009-7322, XP002016020 LIN L-F ET AL: "Mutational studies of streptokinase identify amino acid residues critical to generation of a functional SK-plasminogen activator complex." see abstract 2984	12-14,17 9
P,X	68TH SCIENTIFIC SESSION OF THE AMERICAN HEART ASSOCIATION, ANAHEIM, CALIFORNIA, USA, NOVEMBER 13-16, 1995. CIRCULATION 92 (8 SUPPL.). 1995. I623. ISSN: 0009-7322, XP002016019 LIU L ET AL: "Recombinant streptokinases resistant to cleavage and inactivation by plasmin." see abstract 2985	1,3,4,6,7 9,13

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

'ternational application No.
PCT/US 96/09640

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 7,19
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 7 and 19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inte ional Application No
PCT/US 96/09640

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9407992	14-04-94	AU-A-	5320794	26-04-94
US-A-5011686	30-04-91	NONE		
WO-A-9109125	27-06-91	US-A-	5434073	18-07-95
		AU-A-	4497693	18-11-93
		AU-A-	6954091	18-07-91
		AU-B-	643247	11-11-93
		AU-A-	6965691	18-07-91
		CA-A-	2069085	08-06-91
		CA-A-	2069105	08-06-91
		EP-A-	0502968	16-09-92
		EP-A-	0504241	23-09-92
		WO-A-	9109118	27-06-91
		JP-T-	5502374	28-04-93
		JP-T-	5502375	28-04-93